VOLUME XCII NUMBER 1

THE
BOTANICAL GAZETTE

September 1931

THE GAMETOPHYTES OF THREE SPECIES OF EQUISETUM

ELDA R. WALKER

(WITH FIFTY-FOUR FIGURES)

Introduction

A few years ago the writer (9) reported the occurrence of gametophytes of *Equisetum laevigatum* A. Br. in several localities, and the growing to maturity in the greenhouse of the gametophytes of *E. arvense* L. and *E. telmateia* Ehrh. Since that time the prothallia of these species, as well as those of *E. kansanum* Schaffner, have been found in great abundance in their native habitats. All have also been grown in cultures in a way that made observation possible without injuring them; consequently the same individuals could be repeatedly studied over an extended period. The results of observations on both the wild gametophytes and those grown in culture are here recorded.

Material and methods

Gametophytes of *E. kansanum* have been found on various creek banks in the vicinity of Lincoln, Nebraska, at intervals from 1921 to the present. As the gametophytes of this species cannot be distinguished from those of its close relative *E. laevigatum*, identification of wild specimens depends entirely upon the species growing in the vicinity. Because of this there may be some confusion as to plants taken from nature. All results obtained in all cases were checked by gametophytes grown in culture from spores of known identity.

In the summer of 1922 gametophytes of *E. telmateia* were found in abundance, as follows: at Longbranch, Washington; near the Puget Sound Biological Station at Friday Harbor, Washington; at Olga, Washington; and near Forest Grove, Oregon. At only one place, near Weeping Water, Nebraska, have gametophytes of *E. arvense* been secured from the natural habitat. They were, however, abundant there.

Cultures on sphagnum of *E. kansanum* grew continuously more than two years, those of *E. telmateia* about the same length of time and those of *E. arvense* eight months. All three species were also grown to maturity on soil, but most of the results here recorded were from cultures on sphagnum. The plants from these cultures agreed in all essentials with those grown on soil and those found in their native habitat. Freedom from soil particles simplified both the observations on living specimens and the making of paraffin sections.

The culture method used was a slight modification of that employed by HARTMAN (**6**) in her studies of fern antheridia.[1] Sphagnum was boiled for 45 minutes, and then packed tightly in boiled moist chambers to a depth of 1–2 inches. All surplus water was pressed out. The boiling was only to kill any spores or seeds present, no attempt being made to secure completely sterile cultures. The dishes were covered until cooled, when the spores were shaken directly from the cones on to the surface of the sphagnum by tapping with the finger. The dishes were again covered and placed on the sill of a north window. As no attempt was made to sterilize or wash the spores, fungi and algae invariably appeared. Often fungi started growth with or even before germination of the spores of *Equisetum*. The former were controlled as they appeared with a solution of potassium permanganate. Strong and weak solutions gave equally good results, hence no attempt was made to standardize the solution. Enough crystals of potassium permanganate were added to distilled water to make a deep purple solution. This was poured over the infected areas freely and excess liquid removed. In cases of bad infection the entire culture was submerged in the solution and allowed to stand 5–10 minutes. Then the dish was drained as completely as possible. This treatment was harmless to the gametophytes; in fact,

[1] This method gives equally good results in growing sphagnum from spores.

it seemed of benefit, as well as destroying the fungi. Even cultures not infected with fungi that became sluggish renewed their activity after the addition of potassium permanganate.

As the dishes were kept covered the cultures rarely needed watering. If they became dry, however, they were moistened either with distilled water or with potassium permanganate solution.

Algae occurred in nearly all cultures. At first attempts were made to control them by treating with a solution of copper sulphate, but it was found that cultures containing algae grew better than those without them, so no further control was attempted. BUCHTEIN (3) also found algae harmless to cultures of *Equisetum*. It is possible that they fix nitrogen, as described by ALLISON and MORRIS (1) for certain blue-green algae. While both blue-green and green algae occurred, there was no evident difference in the effects upon the cultures.

The gametophytes in cultures grew normally and produced numerous antheridia, archegonia, and sporophytes. Only in two respects did they differ from gametophytes found in nature. They were slightly more slender and of a little lighter color. During the first year growth was largely in one direction, toward the window, thus producing long narrow thalli. This, however, was an advantage in the observation of sex organs. Eventually in all cases the zone of meristem gradually widened until the gametophytes took a form similar to those found out of doors and described by the writer (9). Often the meristem grew more rapidly at some points than at others, and the thalli came to appear as if composed of radiating lobes.

At frequent intervals specimens were fixed and paraffin sections made for verification of observations on living plants. Formalin-acetic-alcohol (50 per cent alcohol 93 cc., glacial acetic acid 1 cc., neutral formalin 6 cc.) gave the best results, although chromo-acetic and chromo-acetic-osmic solutions were also used.

The use of moist chambers as culture dishes made it possible to examine the thalli with lenses of sufficient power so that archegonia and antheridia could be recognized without removing the plants from the substratum, or in any way injuring them. Thus the same thalli were repeatedly observed. The same individuals were observed at frequent intervals during the period of two years (*E. kansanum*), and many thalli of all species were studied through shorter

periods. Because of the tendency, during the earlier stages, to grow toward the light, entire thalli could be removed and examined as thick sections. After examination, such thalli were fixed and observations were checked in paraffin sections.

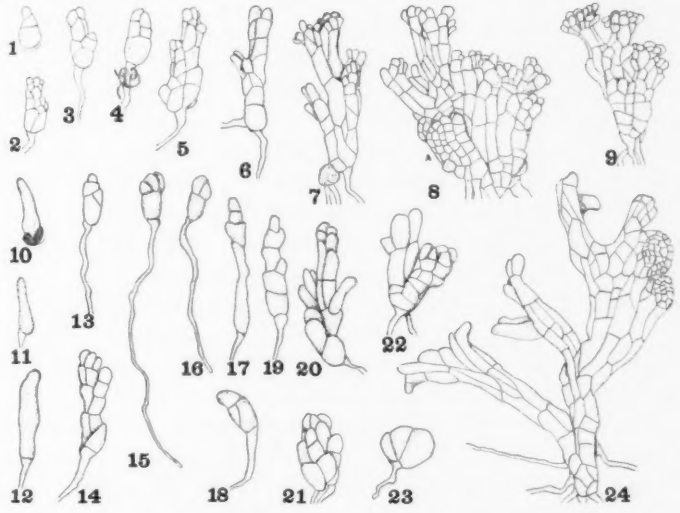
The plants from which the spores used in these studies were taken were identified by Dr. J. H. SCHAFFNER.

EQUISETUM KANSANUM

Gametophytes of this species, as was the case with *E. laevigatum* previously described by the writer (9), have never been found in close proximity to the adult sporophytes. They occur on the narrow floodplain of streams and ponds, whence the spores have been washed from plants growing at higher levels on the prairies. As both *E. laevigatum* and *E. kansanum* commonly occur in Nebraska, and as their gametophytes grown in culture cannot be distinguished, it was inferred that gametophytes found in a vicinity where *E. kansanum* predominates are probably of that species. For this reason the following discussion will deal with gametophytes grown from spores known to be *E. kansanum*. Everything said of this species may correctly be considered as applying equally to *E. laevigatum* previously discussed by the writer (9). They occur in the same type of habitat, have the same peculiar brownish green color, the same development, and the same ultimate form. *E. kansanum* is described because it was the species used in the more extensive studies.

As observed by BUCHTEIN (3) and CAMPBELL (4) for other species of *Equisetum*, the first division of the spore results in a smaller cell, which gives rise to the first rhizoid and to a larger cell from which the thallus is developed. The next division, as well as succeeding ones, may occur in any plane and in any order (figs. 1–9). In some cases, especially in dim light, a filament (figs. 1, 6) of several cells may develop. In such cases further development of the gametophyte takes place from division of the end cells of the filament. More commonly the second division occurs in a different plane from the first (figs. 2, 4, 5, 7, 9), and a massive tissue at once begins to form. After only a few divisions the two parts of the thallus become differentiated, a massive basal region with little or no chlorophyll and upright green branches of characteristic form (figs. 2, 5–9). Early

from the massive base is developed a region of meristem (fig. 8*a*), which continues the growth of the base and gives rise to additional upright green branches, archegonia and antheridia. The meristem widens as it grows; it also grows more rapidly at some points than at others. This causes the thallus to become more or less lobed, and to develop early a tendency to radial form, as described by KASHYAP (**7, 8**) for *E. debile*.



FIGS. 1–24.—Figs. 1–9, early stages in development of gametophytes of *E. kansanum;* ×30. Figs. 10–24, same of *E. arvense* (fig. 24 with two antheridia); ×30.

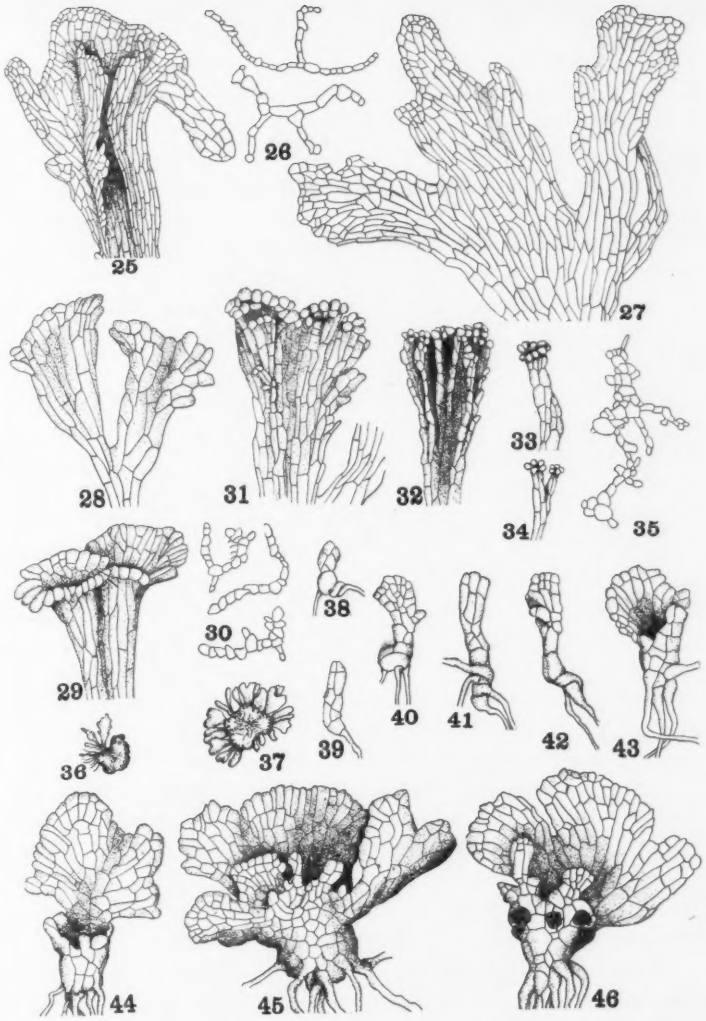
Not only is the first rhizoid long and positively heliotropic as noted by BUCHTEIN and CAMPBELL, but others of the early rhizoids turn in any direction. This often gives a culture one to three weeks old the appearance of being overrun by fungi. Many rhizoids, however, early penetrate the substratum and firmly anchor the thalli.

The persistence of the exospore following germination is variable. More commonly it is not evident after germination (figs. 2–6), although the elaters often remain about the first rhizoid (fig. 4). In some cases, however, the exospore remains until the thallus is of considerable size (fig. 7).

Soon after differentiation of the two body regions, the upright green branches assume the form characteristic of the species. In *E. kansanum* these consist of a column of one to several cells in thickness, at whose top are many small lobes consisting of one or a few cells each (figs. 7–9, 31–34). The tips of these branches, massed closely together, give the surface of the gametophyte a compact, almost granular appearance. Cross-sections of these branches are shown in fig. 35.

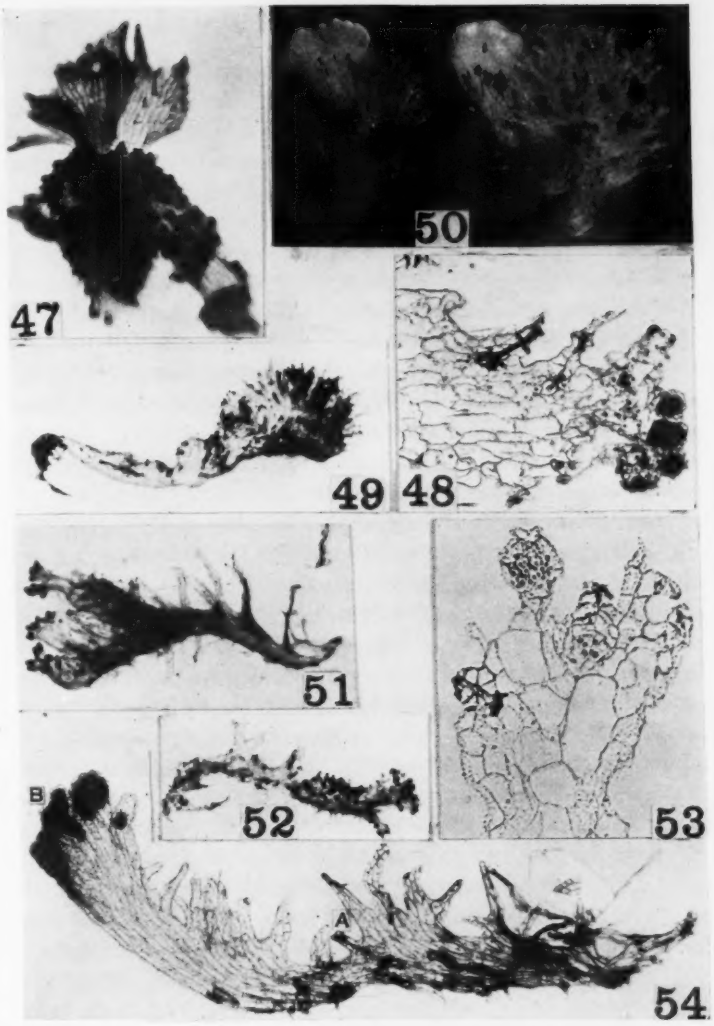
From the time the massive base and region of meristem are differentiated, further growth is variable and apparently dependent upon external conditions. If the planting is thick, so that the thalli are much crowded at once, very little chlorophyll bearing tissue is formed, and few, sometimes no, upright branches appear. Such thalli form antheridia when about 20–30 days old, and soon die. These are the so-called male gametophytes. Normal uncrowded plants, however, or the more vigorous of the crowded ones, form many upright green branches and masses of rhizoids. These (figs. 8, 9) grow 30–40 days before formation of either archegonia or antheridia takes place. The two body regions are well established (figs. 8, 9) before the sex organs begin to be differentiated. On these normal prothallia it is more common for archegonia to appear first, although antheridia first form on some such thalli. Those forming archegonia look in every respect like those forming antheridia, until the sex organs develop. The archegonia, as described by the writer (9) for *E. laevigatum*, occur on the massive tissue between the upright green branches. They are formed in the meristematic rim of the thallus at the same time that the branches are formed, and when mature come to lie between two of these branches. Continued growth of the meristem leaves the archegonia with their accompanying branches distributed over the upper surface of the thallus.

If the first archegonium is fertilized, the growing embryo soon exhausts the young thallus and it does not develop further. Thalli, such as those shown in figs. 8 and 9, normally contain one or more archegonia. If, however, at this stage antheridia develop instead of archegonia, they are formed in the region of meristem (fig. 8*a*), after it has considerably expanded and has ceased to develop green branches. In such cases usually the entire region of meristem becomes a mass of antheridia, and further development does not occur.



FIGS. 25–46.—Figs. 25–35, upright green branches of three species of *Equisetum;* figs. 25, 27, branches of *E. telmateia;* fig. 26, cross-section of same; figs. 28, 29, branches of *E. arvense;* fig. 30, cross-sections of same; figs. 31–34, branches of *E. kansanum;* fig. 35, cross-sections of same; ×30. Figs. 36–46, *E. telmateia:* fig. 36, gametophyte with one archegonium and many antheridia, ×5; fig. 37, thallus from ventral side showing massive base bearing archegonia about periphery, ×5; figs. 38–46, early stages in development of gametophyte of *E. telmateia* (figs. 44 and 45 had archegonia beneath upright lobes; fig. 46 as figs. 44 and 45 and antheridia); ×30.

If some meristem persists after the maturing of the antheridia and a rest period, it may renew its growth and later develop either archegonia (fig. 49) in a mass of upright green branches, or more antheridia after a period of growth. In the plant shown in fig. 49, the base (left in figure) of the thallus is so decomposed that its sex cannot be determined definitely, but a few remnants of upright branches indicate that it had been archegonial. To the right of this darkened base is a typical antheridial region with no branches, few rhizoids, and many old and darkened antheridia. The right half is a continuation of the main axis, and is producing archegonia between the numerous green branches. Below these is the mass of rhizoids characteristic of archegonial regions. The most common condition is for the thallus first to form archegonia among upright green branches, and to continue to do so for some time if fertilization does not occur. After the formation of an indefinite number of archegonia, anywhere from one to a dozen or even more, the meristem region rapidly enlarges and becomes an expanded fan-shaped or club-shaped expansion devoid of chlorophyll and upright branches, and with few if any rhizoids. This expansion bears great numbers of antheridia. Fig. 50 shows two such thalli. Antheridia develop thus through a period of two to four weeks, when growth ceases and much of the thallus dies and becomes dark brown in color. It remains in this condition during a period of two weeks to two months, in which time many individuals die. Those that retain some living tissue then begin to grow, such growths starting from one cell or a group of cells in any part of the thallus. A thallus that has produced archegonia between the green lobes may spread out into many lobes bearing hundreds of antheridia. These lobes and the entire thallus may then become dark brown and appear dead. After as long as two months of dormancy, small groups of cells in the midst of the antheridial mass may begin to grow and develop into green thalli. The new growths in some cases were archegonial and had the characteristic mass of green branches and numerous rhizoids. However, in other individuals such new growths were antheridial. The tendency is for new active growths of this sort to be predominantly archegonial. Had these thalli been grown on soil it would have been impossible to have followed this development, for the older parts were so nearly decomposed that it was only after



FIGS. 47–54.—Figs. 47, 48, 52, gametophytes of *E. telmateia:* fig. 47, thallus with archegonia between green branches and many antheridia, ×13; fig. 48, section of growing tip of thallus with old archegonia and active antheridia, ×38; fig. 52, dwarfed thallus with antheridia, ×10. Figs. 49, 50, gametophytes of *E. kansanum:* fig. 49, thallus that has formed antheridia and is forming archegonia; fig. 50, two gametophytes that have many archegonia between their green branches and numerous antheridia on expanded tip of main axis, ×10. Figs. 51, 53, 54, gametophytes of *E. arvense:* fig. 51, thallus with many old archegonia between branches and many antheridia at tips of main axis, ×10; fig. 53, section of wild thallus showing old archegonia, left and right, with antheridum between them, ×85; fig. 54, elongated thallus with archegonium (*a*) and mass of antheridia (*b*); ×25.

many attempts that a few such were successfully removed from the sphagnum. Many, however, were observed in place. By the time the new growth had matured and in turn formed antheridia, the original archegonial and antheridial regions had usually completely disorganized, and these new shoots appeared as did the original thalli (fig. 50). So large a number of thalli were observed to undergo these reversals in sex (figs. 49, 50) that they are believed to be normal. The small, so-called "male gametophytes" are only abnormal, crowded, and consequently starved thalli which produce a few antheridia and die. If by the death of surrounding thalli, however, a few of these dwarfed "male" plants secure room to grow while they still contain active cells, they develop into characteristic archegonial plants and function as normal thalli.

This alternation in the production of archegonia and antheridia was observed in many individuals in one culture over a period of two years, and for shorter periods in many cultures. During January and February of the first year, and during December and January of the second year, the cultures remained dormant. In each case after this midwinter period of dormancy there followed a period in which only archegonia were being formed by all gametophytes. No living antheridia were present during these periods. At all other times the cultures were predominantly either archegonial or antheridial. During such periods of archegonial dominance some gametophytes formed antheridia, however, and during periods of antheridial dominance some thalli were forming archegonia. Usually only one kind of sex organ was being fomed on a thallus at a time. At the time the reversal was occurring, however, especially in the change from female to male, active archegonia and antheridia often occurred close together on the same thallus. In the reverse change this was not observed, for in all cases following antheridial development there was a period of dormancy, followed by one of growth before archegonia were again formed. There were many cases, however, where a thallus at the same time had several lobes, some of which were archegonial and others antheridial.

No regularity was found in this reversal of sex. In general the dominance of archegonia and antheridia changed about every 20–30 days; however, longer and shorter periods occurred. Still further

there was no set sequence. While more commonly a period of archegonial development was followed by one of antheridial development, gametophytes occurred that rested after archegonial development and again produced archegonia. This often occurred repeatedly and then antheridia sometimes developed. In a large number of the gametophytes death followed development of antheridia. Apparently every living cell was used in antheridial development. Death was rare following the period of archegonial development, but, as mentioned before, new thalli often arise from thalli that are apparently dead and from so little as one cell of such a thallus. In some cases gametophytes were found on whose main axes there had developed archegonia, then antheridia, then archegonia and again antheridia. The older parts were so badly decomposed, however, that it was almost impossible to remove them from the substratum without mutilation. Satisfactory photographs of the few removed were impossible because the older parts were so darkened that they photographed as a black mass. Every imaginable combination and arrangement of archegonia and antheridia occurred. A thallus might fork, one or both lobes forming archegonia; from one of these two lobes might arise, one antheridial and the other archegonial. The other of the two original lobes form either archegonia or antheridia in any sequence. Lobes may arise at any point on either male or female areas, and produce antheridia or archegonia without regard to the sex of the region from which they sprang.

The thalli grew thus, lobing and lobing again in all directions and producing archegonia and antheridia for the entire two years, although the number of individuals was greatly reduced owing to the death of many following the development of antheridia. While many branches arise from gametophytes and become separated by the decomposition of the older parts, this multiplication does not equal the mortality following periods of active development of antheridia. Also multiplication ceases when sporophytes develop. It is only in the case of very large gametophytes like those previously described by the writer (**9**, pl. XXIII, figs. 9, 17) that growth continues after sporophytes begin to develop from them.

Because the cultures were rarely watered, fertilization depended largely upon water condensed on and dropping from the cover of

the dish. This gave occasional sporophytes throughout the period, but following watering they appeared in large numbers.

The thallus starts as a narrow elongated structure with meristem at its tip; it dies in the older parts and decomposes as the meristem region widens, lobing more or less as the case may be. Thus the mature thallus comes to have in general a reniform shape, and is surrounded by a border of meristem. The notch represents the location of the older parts of the thallus.

Various attempts were made to find an explanation for the reversal of sex. It was thought at first that water might be the determining factor. It was found, however, that if of two cultures, both producing archegonia (or antheridia), one was watered and the other not, they would both produce the opposite sex at nearly the same time. With a number of cultures, some watered and others not, all would change approximately at the same time from a predominance of archegonia to one of antheridia and back to one of archegonia. Between periods of watering, cultures often reversed the dominant sex several times. It became evident that water was not the determining factor.

Light also could not have been the cause of reversal, as the cultures remained at all times in the same place and with the same side toward the light. Then too the reversal took place too frequently for length of day to be the cause. What brings about the change was not ascertained, but one fact is suggestive. Archegonia always develop at a time when upright green branches are forming and are active in photosynthesis. This is shown by the fact that they are between these branches and are formed at the time the branches are formed. Also large numbers of rhizoids are formed beneath the archegonial areas (figs. 49, 50). After such a period of active growth and photosynthesis, suddenly the peripheral meristem expands, ceases to form rhizoids, and branch rudiments fail to mature. From this tissue, nearly free of chlorophyll, many antheridia develop. Apparently they develop until the meristem is exhausted, or possibly until the chlorophyll-bearing branches of the archegonial region become old and cease to supply nourishment. Further development of sex organs then does not occur until active chlorophyll tissue is again developed, and this is usually associated with the develop-

ment of archegonia. If archegonia fail to develop, the green branches develop for about the same length of time and then antheridia are formed, as on gametophytes having produced archegonia.

Many sections were examined to determine whether reserve food occurred at one period and not at another. While many individuals contained much starch and others none, no definite relation could be found between this and the production of either antheridia or archegonia. At the time of climax production of antheridia, starch was scarce. It was commonly, although not always, abundant at the beginning of antheridial and during archegonial development. However, its presence at this time was not sufficiently uniform to be of much significance.

Equisetum arvense

The gametophytes of *E. arvense* were found by the writer in but one place, near Weeping Water, Nebraska. These grew on moist clay soil situated in a small bend of a creek. The bank had a northeast exposure and was heavily overhung by trees on the south and west. The bank sloped abruptly from the creek and formed a horizontal surface 1–3 feet above the water level. The gametophytes occurred on the moist soil of this somewhat level surface. The area was covered with adult sporophytes and through it ran a little-used footpath. While the gametophytes were found throughout the area beneath the mature sporophytes, they were larger and more numerous along the path where the soil was packed. Here too was less competition with other plants. The gametophytes were found in the middle of June, and so were well advanced. Very few antheridia and archegonia were present. Most of the thalli bore well developed sporophytes, but some active archegonia and antheridia were present. Fig. 53 shows part of a section of one of these thalli. At the left is an old and darkened archegonium. At the right is an obliquely cut archegonium with a few celled sporophyte in its venter. Between the two is an active antheridium.

In this species, as in *E. kansanum*, development of the gametophyte was followed in plants grown on sphagnum. Here as in the other species a heliotropic rhizoid is formed from the basal cell, while the larger cell resulting from the first division continues to di-

vide in any plane. Subsequent divisions also occur in any plane and without order (figs. 10–23). In some cases (figs. 13, 17, 19) filaments consisting of several cells develop, while in others the formation of a massive tissue starts at once (figs. 15, 16, 21, 23). Also, as in *E. kansanum*, there is early differentiation of the two body regions (figs. 14, 20, 22), the massive base and the upright green branches. Here also badly crowded and dwarfed individuals do not develop normally, but form flattened masses of cells which produce many antheridia, the so-called male plants. These soon die. Thalli having sufficient room for normal growth, however, form the green branches and numerous rhizoids from the massive base on which sex organs soon appear. Some of the green branches of *E. arvense* are flat (fig. 28) and one cell thick, while others are two to three cells thick. Many of these broaden at the top into the form of a funnel (fig. 29).

Of the thalli that have room to grow normally, some may, after developing a few green branches, produce antheridia without having formed archegonia (fig. 24). These also usually live but a short time. If they live, they later produce archegonia.

By far the majority of normal thalli, however, first form archegonia. Anywhere from one to many archegonia develop. Then the meristem suddenly ceases to form green branches, expands, and usually turns upward (figs. 51, 54). Masses of antheridia are formed in these upturned extensions of the main axis. The thalli shown in figs. 51 and 54 had produced an archegonium at the base of nearly every green upright branch. The older parts of the thalli show the darkening characteristic of old tissue, while the upturned tips show the active antheridia. The antheridia of *E. arvense* protrude from the surface of the thallus more than do those of the other species (figs. 51, 54). Fig. 54 shows a long thin thallus of this sort, produced by growth toward the window. At *a* is an old archegonium. Younger ones, out of focus in the photograph, were present between other branches, while many antheridia show in the youngest tissue (*b*) at the extreme left.

Six weeks after planting, many of the flat crowded gametophytes had produced antheridia in large numbers and were dying. The less crowded larger gametophytes had the usual upright green branches. Most of these had developed no sex organs. A few bore only an-

theridia, while a few had produced one archegonium and were developing antheridia. All active thalli had upright green branches. When eleven weeks old practically all plants that formerly had borne antheridia were dead. The remaining plants had grown toward the light and had attained lengths up to 5 mm. and widths up to 3 mm. Nearly all bore many archegonia, and many were producing antheridia at the tips of the branched upturned axis (figs. 51, 54). Many others continued to form archegonia. After a period of rest, as in *E. kansanum*, new growths started from all surviving thalli. These were archegonial or antheridial without regard to the sex of the region from which they sprang; however, this renewing of growth took place to a much less extent than in *E. kansanum*. In most cases the gametophytes grew vigorously until one of two things happened. When fertilization took place and embryo development began, the gametophyte became exhausted and further growth ceased. If fertilization did not occur, development of archegonia continued for a greater or less time, but eventually antheridia formed at the tip of the axis. The meristem was all used in their formation and the thallus died. Very few such plants survived again to form archegonia. At the end of eight months all gametophytes had died, either from development of sporophytes or from the production of antheridia.

Equisetum telmateia

The gametophytes of *E. telmateia*, like those of *E. arvense* but unlike those of *E. kansanum* and *E. laevigatum*, previously reported by the writer (**9**), have been found in close proximity to the plants from which the spores were discharged. Like the other species studied, these gametophytes were found only on clay soil; unlike the others they occurred only on vertical surfaces.

At Longbranch, Washington, they were found on a vertical bank near a waterfall, where there was an outcropping of clay. The bank sloped to the east and was heavily shaded by trees. Mature sporophytes overhung the bank. Near the Puget Sound Biological Station at Friday Harbor, Washington, gametophytes in all stages, from a few cells to 3 mm. in diameter (figs. 38–46), were found during the period from June 24 to July 25. These grew on the verti-

cal walls of a pit in which a water reservoir stood. The pit was in the northeast side of a hill. The growth of gametophytes was so dense as completely to cover the clay soil. They afforded ample specimens for study during this period. In this species all illustrations of developmental stages were made from these specimens found in nature. Mature sporophytes overhung the edges of the pit above the gametophytes. In the same vicinity gametophytes were found also, on the vertical sides of a small ditch in the midst of an area covered by sporophytes. Here the soil contained some sand. The walls of the ditch were deeply shaded by masses of *Equisetum* and ferns. On June 23 of the same year many gametophytes varying in size up to 3 mm. in diameter were found at Olga, Washington. These were on a steeply sloping bank on the shore of Puget Sound. However, they occurred only on vertical surfaces of clay outcroppings. The ground was kept moist by seepage from springs above. The exposure was east and shaded by trees. Many adult plants were found higher on the bank. During July and August large numbers of mature gametophytes, many of them bearing sporophytes 2–10 cm. high, were found near Forest Grove, Oregon. These were in a deep wide ditch running east and west and draining water from a hill covered with adult sporophytes. The ditch had been roughly dug. Its walls were of clay and vertical, and in its bed, which carried only a small amount of water, were many elevations extending 15–30 cm. above the water level. Gametophytes of varying size up to 7 mm. in diameter were thickly scattered over all moist vertical surfaces, but while the horizontal surfaces of the elevations in the bed were well moistened, no gametophytes were found on them.

The gametophytes of this species were thus found at five different places in great abundance. In all cases they were on only vertical surfaces of clay soil. All were in places where more or less shade occurred during the greater part of the day. In all locations there was a constant supply of moisture. Gametophytes grown on sphagnum agreed in all essential details with those found in the native habitat. Here they grew best on vertical surfaces, but they also did well on horizontal surfaces that were not unduly wet. It would seem that well drained moist surfaces and reduced light are essential for the growth of these prothallia.

On the germination of the spores, as is the case in the preceding species, the first divisions occur in any plane and are followed by other divisions without order (figs. 38–43). The first rhizoid arises from the lowest cell and is heliotropic. Other rhizoids arise from any of the early thallus cells and turn in any direction. As in *E. kansanum* and *E. arvense*, there is early differentiation of the two body regions, the massive base and the upright green branches. The base, as in other forms, soon develops a meristem which widens and often lobes until it forms a more or less circular body, the upper surface of which is covered with the upright green branches. Its under surface is thickly covered with rhizoids. The upright branches of this species are different from those of the other species studied. They are broad and usually one cell thick, except at points from which lateral platelike lobes arise (figs. 25–27, 43–47). They resemble somewhat the flat type of branches found in *E. arvense*. Because of the broad flat branches, early stages (figs. 44–46) might be mistaken for fern prothallia. At the stage shown in fig. 37, when seen from above, a gametophyte looks like a clump of fern prothallia standing more or less erect from the base. In older plants the basal axis may become much more lobed than is shown in fig. 37. In all plants taken from the native habitat, however, the circular form is roughly maintained. Plants grown in culture show more lobing of the base than do those growing in the open, but the lobes tend to radiate in such a way as to give eventually a circular appearance.

As in the preceding species, crowded cultures produce many slender, dwarfed, flat plants which give rise only to antheridia (fig. 52) and soon die (the so-called male gametophytes). The less crowded and more vigorous individuals form the massive base and upright green branches (figs. 44–46). In all cases examined, as soon as the first green branch is developed (figs. 43–46), an archegonium forms between it and the rudiment of the next green branch. It lies beneath the branch so that it is not readily seen. Often but one archegonium forms. If this is fertilized the gametophyte develops no further. If fertilization does not occur the meristem may at once enlarge and produce a mass of antheridia (figs. 46, 47). These are commonly known as "male thalli." It is not strange that the one archegonium between the green branches is easily overlooked.

Two months after planting, all normal gametophytes contained archegonia, and two weeks later sporophytes were visible. Most of these had developed from the first archegonium formed by the thallus. At an age of three months nearly all of the early crowded gametophytes had died, but many antheridia were forming on the meristem of thalli that had produced one or more archegonia. Other gametophytes continued to develop archegonia for some time before antheridia were produced. At four months some gametophytes had attained a diameter of 8 mm. and sporophytes 5–8 cm. high were present. The long, thin upright branches are loosely set and the large archegonia are conspicuous between them.

In a majority of plants more than one archegonium develops, and their formation may continue indefinitely, giving rise to the so-called female plants (figs. 37, 44–45). In all cases observed in culture, however, if fertilization does not occur, these archegonial thalli eventually develop antheridia from the meristem of the main axis or of some lateral branch of it. Fig. 48 shows a vertical section of such a thallus. Parts of two archegonia show, one darkened by age. The one nearer the antheridia still shows the egg, although the neck is darkening and shriveling. At the right is the antheridial region.

While this species did not grow as vigorously in culture as did *E. kansanum*, a limited number of thalli were observed over a period of two years. They passed through the same changes of sex as described for *E. kansanum*. As in that species, it is rare to find active archegonia and antheridia on a thallus at the same time, but repeated reversal of sex, both from female to male and back to female, were observed. Except for some early dwarfed individuals, all bore at least one archegonium before forming antheridia.

Only two cases were observed where antheridia were formed without the development of a more or less massive tissue. In one of these the thallus was but two cells thick; in the other case two antheridia were formed at the tip of an upright green branch.

Discussion

It is interesting to find that the gametophytes of the three species of *Equisetum* studied differ in the type of their habitat and in the form of their green branches. The branches are so characteristic

that the thalli can readily be distinguished by them; nevertheless their general mode of growth and reproduction are the same. All agree with *E. laevigatum*, previously described by the writer (9), and *E. debile*, described by KASHYAP (7), in being characteristically monoecious. The thalli that have usually been considered male plants are only the crowded and greatly dwarfed individuals. Those that have been considered female are the normal individuals which form both archegonia and antheridia. In all the species these normal plants characteristically produce first archegonia and then antheridia. Not only was this found to be the case in plants grown in culture, but also in those found in their native habitat. From these studies it becomes evident that the gametophytes of *E. laevigatum* previously described (9) were found just when the change from development of archegonia to that of antheridia was taking place, and hence the monoecious nature of the thalli was evident.

The occurrence of antheridia and archegonia on the same thallus was observed by BUCHTEIN (3), but the complete significance of his observations was not appreciated. Many who have worked with gametophytes of *Equisetum* have observed an occasional antheridium on a female plant, and vice versa. Probably the reason this has not been more commonly observed and its significance realized is that the same individuals have not been under observation for extended periods. Plants grown in culture just until archegonia begin to develop would give the impression of being dioecious. The mature ones taken from the native habitat at any one time might or might not show the two sexes. Only in large and vigorous thalli, such as those previously described (9), will growth and development of sex organs be continued after sporophytes are formed.

BUCHTEIN (3) observed that changes of light did not bring about changes in sex. He found, however, that crowded sowings produced few female plants and thin sowings produced many. He found that spores grown in pure water or on washed sand produced only male plants, while female plants removed from loam to sand produced only antheridia. He concluded that nutrition must in some way be the determining factor. BOWER (2) showed that even among homosporous ferns, whose thalli are recognized to be monoecious, gametophytes starved by crowding become reduced and bear only anther-

idia. CZAJA (5) also found in homosporous ferns that monoeciousness is normal, but that either sex may be suppressed so that the thalli may become dioecious. He considers this due to external factors.

It is evident that *Equisetum* agrees with the homosporous ferns in that its gametophytes, which are normally monoecious, may under crowded conditions of growth appear dioecious. As found commonly in nature and in cultures, the large gametophytes overrun many other thalli which are crowded beneath them and produce only antheridia. In cultures it was found that if the large thallus dies or is removed, some of these crowded thalli take on vigorous growth and begin to develop archegonia.

Summary

1. Gametophytes of *Equisetum kansanum*, *E. telmateia*, and *E. arvense* were found in large numbers in their native habitats, each of the three species having a characteristically different one.

2. All three species were grown to maturity on sphagnum, and such plants agree in all essential points with those found in their natural habitat.

3. The gametophytes of all species agree in their early development and at maturity in having a massive base, a meristematic rim, and upright green branches. The upright green branches are characteristically different in each of the species.

4. In all species archegonia and antheridia are borne on the massive tissue of the main axis or of its lobes. Only rarely do antheridia occur on the upright branches.

5. Normal thalli are monoecious. Dwarfed and starved plants produce only antheridia.

6. Archegonia develop from meristem that is forming upright green branches and many rhizoids, and at maturity lie between these branches.

7. Antheridia develop from meristem which is forming reduced branches or none, and few or no rhizoids. At maturity they occupy expanded chlorophyll-free regions. Following development of antheridia, the thallus rests for a time before again developing archegonia.

8. On normal thalli it is usual for archegonia to develop first, then antheridia; still later archegonia may again develop, followed by antheridia.

9. Development of antheridia brings about exhaustion of the thallus and results in the death of many plants. Fertilization and the consequent development of an embryo also bring about cessation of growth in the thallus. Exceptions occur only in case of very large thalli, which may continue growth with the formation of additional sex organs, until several sporophytes have developed.

10. In degenerating thalli any cell or group of cells may return to active growth and develop lobes which function in all respects as the original thallus.

11. Cultures of *E. kansanum* and *E. telmateia* have been under continuous observation for two years; those of *E. arvense* for eight months.

The writer is indebted to Professor J. H. SCHAFFNER for identification of the plants from which the spores were obtained for cultures, and to Professor T. J. FITZPATRICK for careful reading of manuscript and proof. For many courtesies the writer is also indebted to Dr. T. C. FRYE and the Puget Sound Biological Station, where much of the work on *Equisetum telmateia* was done, and to Dr. G. R. LARUE and the University of Michigan Biological Station, where the paper was completed.

UNIVERSITY OF NEBRASKA
LINCOLN, NEB.

[*Accepted for publication December 10, 1930*]

LITERATURE CITED

1. ALLISON, F. E., and MORRIS, H. J., Nitrogen fixation by blue-green algae. Science **71**:221–223. 1930.
2. BOWER, F. O., The Ferns. Vols. III. University Press. Cambridge, England. 1923–1928.
3. BUCHTEIN, O., Entwicklungsgeschichte der Prothallium von *Equisetum*. Bibliotheca Botanica **8**:1887.
4. CAMPBELL, D. H., The structure and development of mosses and ferns. Macmillan Co. New York. 1918.

5. CZAJA, A. TH., Über Befruchtung, Bastardierung und Geschlechtertrennung bei Prothallien homosporer Farne. Zeitschr. Bot. **13**:545–589. 1921.
6. HARTMAN, M. ELIZABETH, Antheridial dehiscence in the Polypodiaceae. BOT. GAZ. **91**:252–276. 1931.
7. KASHYAP, S. R., The structure and development of the prothallus of *Equisetum debile* Roxb. Ann. Botany **28**:163–181. 1914.
8. ———, Notes on *Equisetum debile* Roxb. Ann. Botany **31**:439–445. 1917.
9. WALKER, ELDA R., The gametophytes of *Equisetum laevigatum*. BOT. GAZ. **71**:378–391. 1921.

DEVELOPMENT OF THE MACROGAMETOPHYTE AND EMBRYO OF DAUCUS CAROTA

H. A. Borthwick

(WITH THIRTY-TWO FIGURES)

Introduction

The work reported in this paper, while dealing mainly with embryogeny of *Daucus carota* L., also includes some observations on the structure of the female gametophyte and on the development of the pollen tubes. The embryogeny of the Umbelliferae has not been investigated extensively, but the few species which have been examined seem to have certain features in common. In all of them the embryo develops into an unusually long structure before longitudinal divisions occur. The sequence in which the cells divide is extremely variable among individuals of the same species. Souèges (17), who has made a careful study of *Carum carvi* L., finds that its embryonal development is not essentially different from that of certain members of the Solanaceae (15) and the Rubiaceae (16). Further critical studies of embryogeny of other members of the Umbelliferae, therefore, have seemed desirable for comparison with the few which have been studied.

The carrot (*Daucus carota*) was selected for this study because it was thought that such an investigation might throw light on practical problems connected with seed germination. These studies of *Daucus* show that a large percentage of the seeds fail to germinate. Examination of these seeds shows that the failure is due to defective embryos. While considerable information has been obtained during this investigation on the causes of the failure of embryos to develop, this phase of the problem will be discussed in a later paper.

Materials and methods

The carrots from which material was collected were of the Chantenay variety. Most of the plants were grown at Davis, California; others were grown by C. C. Morse and Co. near Clarksburg, California. The material was killed and fixed in a solution made as fol-

lows: solution A, 4 parts of commercial 40 per cent formalin and 1 part water; solution B, 1 gm. chromic acid and 10 cc. acetic acid in 90 cc. of water. Equal parts of the two solutions were mixed at the time they were used. Material showing stages in the development of the female gametophyte and the early embryogeny were stained with Haidenhain's iron-alum-haematoxylin. Delafield's haematoxylin was found very satisfactory for later stages in embryonal development. Pollen tubes were well differentiated with a combination of Haidenhain's haematoxylin and resorcin blue. The points in which this special technique for staining pollen tubes differs from that of BRADBURY (3) will be described in a later paragraph.

Literature review

A survey of the literature dealing with the morphology of *Daucus carota* shows that comparatively little has been done on that part of the life cycle included under the title of this paper. The most extensive investigation is that of HÅKANSSON (7), who describes the development of embryo sacs of a great number of genera of Umbelliferae, including *Daucus*. He reports that in *Daucus* a single archesporial cell gives rise to four macrospores in a rather limited nucellus. The embryo sac, which is at first small, enlarges rapidly and ruptures the nucellus transversely. One or more nucellar cells frequently persist next to the tip of the expanding embryo sac. The chalazal portion of the ruptured nucellus is conspicuous in the lower part of the ovule, and in it the antipodals remain evident long after fertilization. HÅKANSSON makes no reference to the details of fertilization, and merely states that the embryo has a long suspensor. MEZ (12), generalizing on the mature embryos of Umbelliferae, states that the calyptra of the root is always well developed and the plumule always absent. He says that the embryo of *Daucus* is elongate and has broadly linear cotyledons that are as long as the radicle.

Although the macrogametophytic development of *Daucus* has been briefly described by HÅKANSSON, other members of the family have been investigated more thoroughly in this respect. HÅKANSSON, in his general discussion of the family, states that archesporial cells function directly as macrospore mother cells without giving rise to any parietal tissue. He found, however, that in a number of

members of the family a multicellular archesporium is produced, a fact which BEGHTEL (2) also reported for *Pastinaca*. In nearly all cases examined HÅKANSSON found that four macrospores are produced, and that the inner gives rise to an 8-nucleate embryo sac in the usual manner. This differs from BEGHTEL's account of *Pastinaca*, in which he says, "It appears that the outer megaspore invariably functions." He bases this statement on the fact that in the chalazal end of the nucellus there is a mass of dark staining, disintegrating tissue which he assumes to be the disintegrating spores. This interpretation is open to question. It seems more probable that this dark staining material is not disintegrating spores but is the tissue found in many umbelliferous plants which HÅKANSSON regards as similar to the hypostase of the Onagraceae. Nothing particularly unusual has been reported concerning the macrogametophytic development in the family, aside from the early rupture of the nucellus and the rapid enlargement of the embryo sac at that time. HÅKANSSON finds a more or less striated appearance in the micropylar part of the synergids, but states that a filiform apparatus is not present.

The details of embryo development are not well known for the family as a whole. HÅKANSSON shows a few figures of early development in *Foeniculum vulgare*, *Carum carvi*, and *Anethum graveolens*, and records the fact that HEGELMAIER (8) has studied similar stages of *Petroselinum*, *Bunium bulbocastanum*, and *Eryngium bulbosum*. In all of these species it is apparent that the 4-celled embryo is linear. The next divisions are also transverse in many of them, so that filamentous embryos eight or more cells long, in which no longitudinal divisions have taken place, are common. In *Eryngium yuccifolium*, JURICA (9) reports the presence of a long suspensor and of considerable diversity in the sequence of cell division, but his figures are all of rather advanced embryos. TANFANI (19) says that embryos, 10–14 cells in length without longitudinal divisions, are not uncommon in the Apiaceae.

In all of the papers mentioned development of the embryo was not the main problem, and consequently was not investigated at all thoroughly. SOUÈGES (17), working with *Carum carvi*, has recently published the most detailed embryological study yet made of the Umbelliferae. In this species he finds that a linear file of four cells

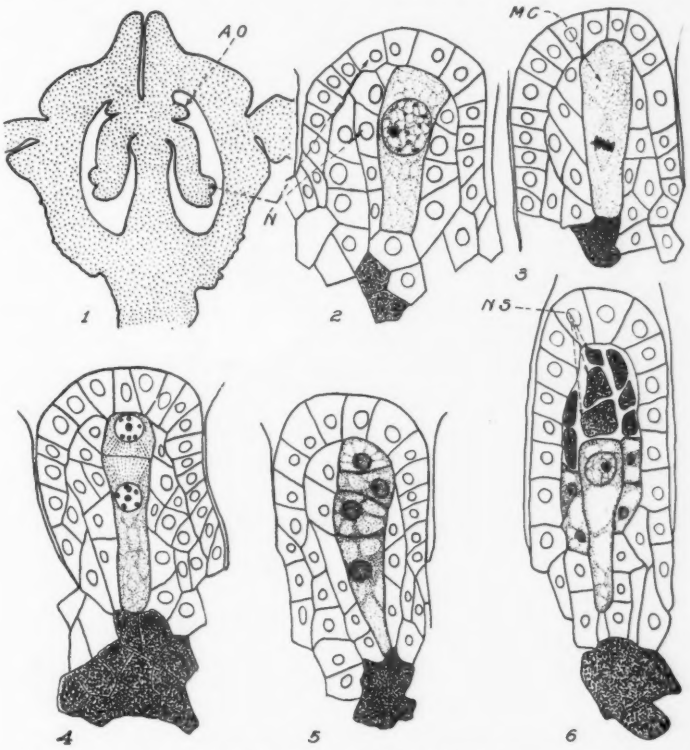
is invariably formed, after which the planes of division may vary. The two cells nearest to the micropyle almost always divide transversely while the other two behave in one of three ways. Both may divide longitudinally; the terminal one of the two may divide transversely and the other longitudinally; or both may divide transversely. The resulting 8-celled embryos are respectively six, seven, and eight cells long. Each of these 8-celled types is figured by SOUÈGES (17), and he shows a number of later stages in the development of each type into a mature embryo. Although these three types of 8-celled embryos are different, the mature embryos produced from them are essentially alike. In summarizing his work, SOUÈGES points out that there is but one type of 4-celled embryo which gives rise to these three different types of 8-celled embryos. He finds, moreover, that each cell in the 4-celled stage gives rise to certain definite parts of the mature embryo, regardless of variation in the sequence or planes of division. He concludes that in mature embryos of *Carum carvi* the cotyledons arise from the distal cell of the 4-celled embryo; the hypocotyl from the cell immediately below it; the root cap primordium and a portion of the suspensor from the next lower cell; and the remainder of the suspensor from the proximal cell of the four. SOUÈGES has made similar studies of representatives of a great many families, and his generalizations based on these studies will be discussed later.

Macrogametophytic development

At the time the archesporial cell first becomes evident in the nucellus, the ovule is pendent in the locule and the single integument has just begun to appear (fig. 1). Its nucellus consists of a small cylindrical mass of tissue which, when viewed in cross-section, varies from five to seven cells in diameter. At its chalazal end there can usually be found a number of dark-staining cells which appear to be in a state of disintegration. These become even more conspicuous a little later. This tissue is the hypostase to which HÅKANSSON refers and which BEGHTEL apparently confuses with disintegrating sporogenous tissue in *Pastinaca*.

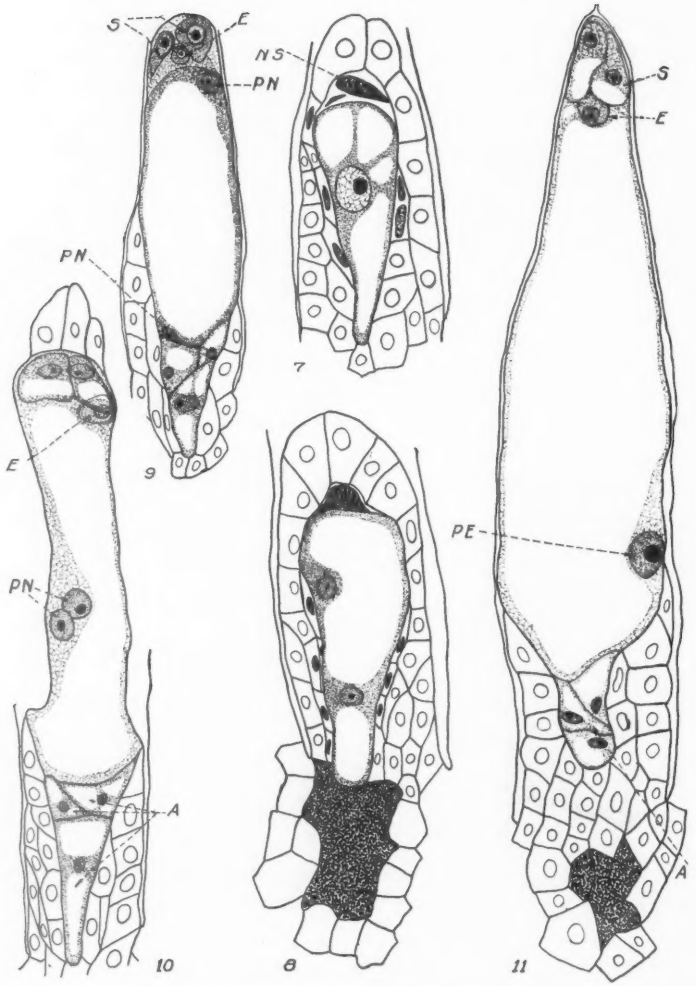
The single archesporial cell functions directly as a macrospore mother cell, a feature reported by HÅKANSSON as characteristic of

the family. Nothing unusual was found in the development of the macrospores (figs. 2–5). The mother cell gives rise to a linear tetrad with cytokinesis following each mitosis. The embryo sac arises from the innermost macrospore, which enlarges rapidly at the expense of



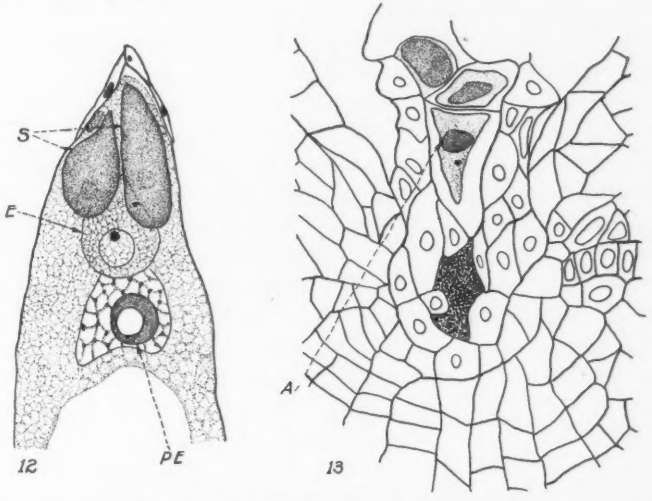
FIGS. 1–6.—Fig. 1, longitudinal section of flower at time of macrospore mother cell formation: *AO*, abortive ovule; *N*, nucellus; ×80. Figs. 2–6, stages in macrospore formation: *MC*, macrospore mother cell; *NS*, non-functional spores; ×750.

the other three (figs. 6, 7). The 8-nucleate condition is attained while the embryo sac is still only a fraction of its final size. As the macrogametophyte approaches maturity, the two polar nuclei migrate from opposite ends to the center of the embryo sac and fuse with each other (figs. 8–11). Fusion takes place well before fertiliza-



FIGS. 7–11.—Successive stages in embryo sac formation: *S*, synergids; *E*, egg; *PN*, polar nuclei; *NS*, non-functional spores; *A*, antipodals; *PE*, primary endosperm nucleus; ×750.

tion. The primary endosperm nucleus thus formed then migrates to a position immediately under the egg apparatus where it remains until the time of fertilization (fig. 12). It is large and contains an enormous nucleolus. The egg at this time is somewhat elongate, vacuolate in the upper part, but with rather dense cytoplasm and a small nucleus at the lower end. The synergids contain abundant cytoplasm with small vacuoles in their lower ends and filiform structure at their apices. This last feature will be discussed more fully in another paragraph.



FIGS. 12, 13.—Micropylar and antipodal ends of embryo sac ready for fertilization: *S*, synergids; *PE*, primary endosperm nucleus; *A*, antipodals; ×750.

During enlargement of the embryo sac the nucellar cells lateral to the non-functional macrospores disintegrate (figs. 6, 7). The nucellar epidermis remains intact, however, until it is finally ruptured transversely by the enlarging embryo sac (figs. 9, 10). This usually occurs at about the time the sac reaches the 8-nucleate stage. The nucellar cells which are thus torn loose from the chalazal part of the nucellus may be seen for a time at the tip of the egg apparatus (fig. 10), but they soon disintegrate. The chalazal portion of the nucellus, however, persists much longer. In it the three antipodals may

be recognized until after fertilization (fig. 13). They usually have a triangular arrangement, with one somewhat elongate directed toward the chalaza and the other two side by side above it. After the nucellus is ruptured the embryo sac lies in direct contact with the inner epidermis of the integument. The ovule during these changes enlarges rapidly and the embryo sac keeps pace with this enlargement.

Although HÅKANSSON states that a filiform apparatus of cellulose is not found in the Umbelliferae, I find evidence to the contrary in *Daucus*. The staining reactions of the somewhat striated, caplike apices of the synergids in the mature macrogametophyte give a distinct cellulose reaction. With safranin and light green, for example, the apices of the synergids stain green with small spots of red scattered throughout. These red spots become progressively larger and more abundant back from the apex. The green-staining continuous phase I interpret as being composed of cellulose, while the red-staining discontinuous portion is the cytoplasm inclosed in the minute cellulose chambers. With Haidenhain's haematoxylin and a light counter-stain of orange G, the tips of the synergids stain orange with small dark spots in them which have taken the haematoxylin. In this case the haematoxylin has stained the cytoplasm and the orange G the walls. Both of these staining reactions indicate the presence of cellulose in the tips of the synergids, but in order to prove the point more definitely microtome sections were treated with chloroiodide of zinc according to ARTSCHWAGER'S (1) method. Synergids treated in this way show a definite blue color in that portion which takes the light green or the orange G. This shows that a definite filiform apparatus of cellulose is produced in *Daucus*. Its structure appears to be similar to that described by HABERMANN (6), but it is not developed as conspicuously as in the plants which he studied.

Pollen tube development

Preparations stained in the usual manner showed pollen tubes in such great abundance along the funiculus of the ovule and about the micropyle that it was thought desirable to devote special attention to their development. While the tubes could easily be traced where they were free in the locule, such was not the case in other

parts of the pistil. After a number of trials of various stains, resorcin blue, which was first used by TSVETT (**20**), was selected as the most satisfactory for tracing the path of the tubes through the tissues of the style. Paraffin sections, 10 μ thick, were stained in Haidenhain's iron-alum haematoxylin and counter-stained in a 0.5 per cent solution of resorcin blue for 1–24 hours. They were then washed quickly in water and mounted in Apathy's medium directly from the water. Mounting in this medium, made up as suggested by LEE (**10**), is much more satisfactory than mounting in balsam, since most of the resorcin blue is removed from the sections when they are passed through the grades of alcohol and xylol into balsam. These preparations dry quickly, in fact even quicker than those mounted in balsam. Slides prepared in this way have been kept for several months with no evidence of deterioration except for a slight amount of crystallization of the mounting medium around the edges of the cover slip, an effect that could probably have been prevented by sealing the mounts with gold size or some other cement. The only tissue in the preparations which stains blue with this method, other than the callose portions of the pollen tubes, is the degenerating part of the two abortive ovules which are situated almost directly above the point of attachment of the two fertile ones (fig. 14 *h*). Resorcin blue was found to be a somewhat satisfactory stain when used alone, since it stains the protoplasts of cells a reddish brown and the walls of the tubes a bright blue. Better definition is obtained, however, by using it as a counter-stain after the haematoxylin. Other stains, such as aniline blue and rosolic acid, were used but were found less satisfactory than resorcin blue.

At the time of pollination the pistil has the structure shown in fig. 14. The two cylindrical styles are 2–3 mm. long and diverge slightly from each other. The lower portions of the styles are grooved on their inner faces, a feature best seen in cross-sections cut at this level (fig. 14 *a*, *b*). Lower down the two grooves form a single slot-like canal in the fused bases of the styles (fig. 14 *c*) that communicates laterally with the locule of each carpel. There are three vascular bundles extending about two-thirds of the length of each style, one opposite the groove and the other two lateral to it. Between these three bundles and the grooved side of the style is a con-

ductive tissue composed of elongated cells. The upper end of the conductive tissue is the stigmatic surface. In each locule there is an

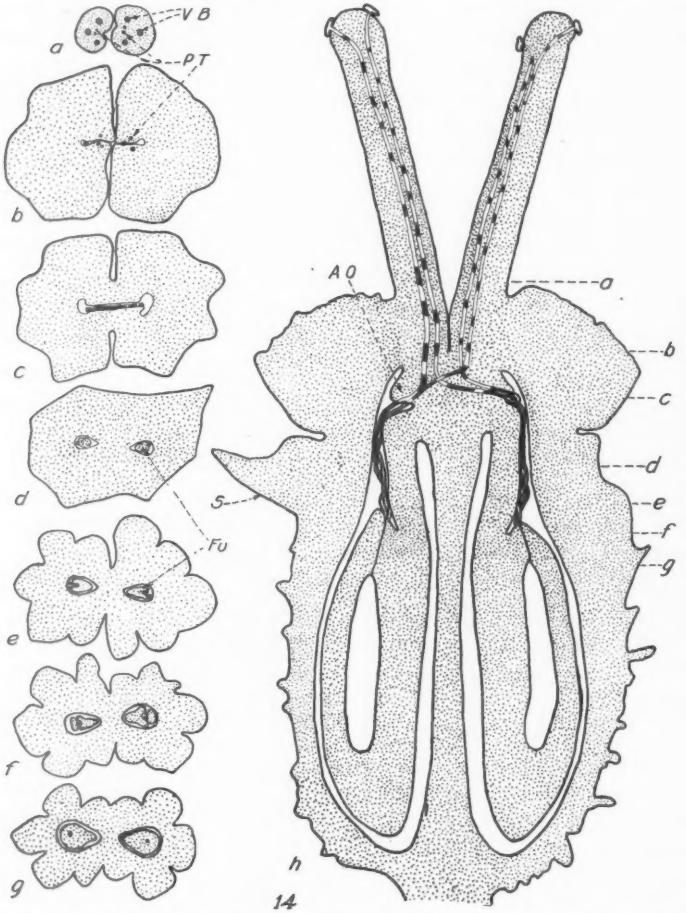


FIG. 14.—*a-h*, cross and longitudinal sections of flower showing course of pollen tubes diagrammatically: *a-g*, cross-sections taken at levels *a-g* of *h; VB*, vascular bundles; *PT*, pollen tubes; *Fu*, funiculus; *S*, sepal; *AO*, abortive ovule; *a-g*, ×16.5; *h*, ×30.

abortive ovule, and below it a larger functional one with a long funiculus and a single integument. Near its upper end the funiculus is

approximately cylindrical in cross-section (fig. 14 *d*), but lower down it has a groove which lies almost directly above the micropyle (fig. 14 *e*, *f*). Funicular cells along this groove have dense protoplasts and appear to be secretory in nature.

The pollen grains, germinating on the stigma, send out tubes that grow between the cells of the stigmatic surface and intercellularly down through the conducting tissue (fig. 14 *a*). Toward the basal part of the styles the path of the tubes lies close to the stylar groove (fig. 14 *b*), and at a slightly lower level the tubes emerge from the interior of the style and grow along its grooved surface. At the level of the transverse canal (fig. 14 *c*) they may either cross over from one carpel to the other or continue downward in the same carpel. Both possibilities in the growth of pollen tubes have been observed repeatedly.

When the tubes have reached the locule they grow around or over the abortive ovule and to the point of attachment of the fertile one. From here their course is downward along the grooved surface of the funiculus to the micropyle. CAMMERLOHER (**4**), studying the ovules of a number of Umbelliferae, including *Daucus*, also observed this secretory funicular tissue along which the tubes grow, and states that it serves to conduct the pollen tubes to the micropyle. The tubes usually bend away from the funiculus as they approach the ovule, and pass directly into the micropyle and down it to the apex of the embryo sac. Sometimes, however, they follow down along the funiculus to the point where it joins the ovule and then up the surface of the integument to the micropyle. In two or three cases tubes were seen to branch as they entered the micropyle. Although this is not common in angiosperms, DAHLGREN (**5**) mentions a number of cases where it has been noted. While several tubes have been observed entering one micropyle, it is not uncommon to find others which have failed to reach the micropyle, growing for a short distance along the outer surface of the integument.

After entering the embryo sac the pollen tubes grow between the two synergids. The filiform apparatus is ordinarily left intact and can be identified for some time after fertilization. The cytoplasmic portion of the synergid, on the other hand, disintegrates soon after the entrance of the pollen tube. It is not unusual to find pollen tubes

growing past the egg apparatus. In one case a tube was traced for nearly one-third the length of the embryo sac. In this particular ovule fertilization had already occurred, and the endosperm consisted of about sixteen free nuclei distributed about the periphery of the embryo sac. The tube did not penetrate the cytoplasm of the sac but grew between it and the inner surface of the integument.

Although STRASBURGER (**18**) observed the occurrence of plugs in pollen tubes, the callose nature of these plugs and of the inner lining of the pollen tube wall was first thoroughly discussed by MANGIN (11). The distribution of the callose in pollen tubes of *Daucus* is not the same throughout their entire length. Near the stigma and in the upper half of the style, the walls of the tubes may stain a faint blue only, or they may take up sufficient stain to stand out sharply. The plugs, on the other hand, are conspicuous and give a strong callose reaction. They differ greatly in size and shape, and vary all the way from globular masses adhering to one side of the wall to transverse septa. They are usually 3–10 μ in length and are spaced at intervals of about 25 μ.

Toward the base of the style the plugs are longer and lie closer together than in the upper part. Lateral walls between plugs also show a callose reaction more frequently than at higher levels in the style. Portions of the tube within the locule and micropyle have fewer plugs, but the entire wall of the tube is frequently much thickened with callose. In some cases the lumen is reduced to one-third or one-fourth the diameter of the tube, and here and there it appears to be almost completely closed.

In older material, in which the embryo was 2-celled and the endosperm about two layers of cells thick, the tubes along the funiculus were found to have lost their capacity to stain with resorcin blue, although they could still be recognized in the unstained condition. In the micropylar region the tubes still stained blue but appeared somewhat disorganized. In the stylar tissue, however, the plugs appeared to stain even more conspicuously than at the time of fertilization.

Embryogeny

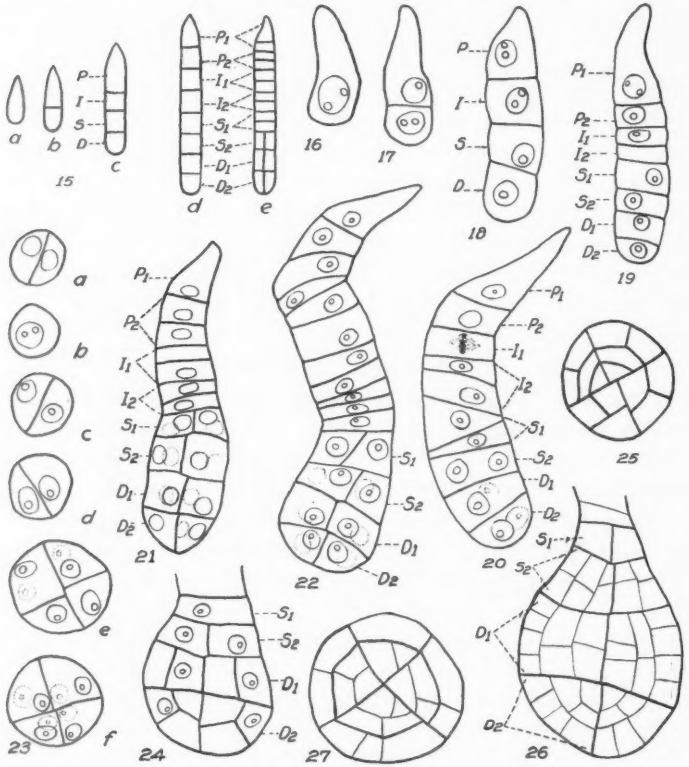
Although fertilization was not observed, the stages subsequent to it were found in abundance. Division of the primary endosperm nu-

cleus takes place soon after fertilization, and the number of nuclei becomes considerable before the zygote divides. These nuclei are numerous in the upper part of the embryo sac around the zygote; elsewhere they are confined to the periphery of the sac. The first few divisions of endosperm nuclei are nearly simultaneous, but toward the end of the free nucleate stage their order becomes irregular. The multinucleate endosperm becomes cellular about the time the embryo is 2-celled. After this the number of endosperm cells increases rapidly by cell division. The central cavity within the immature seed is soon nearly filled with endosperm tissue, while the embryo still contains only a few cells. Part of this tissue is digested as the embryo elongates and pushes into the central part of the seed. At maturity the seed is entirely filled with rather thick-walled endosperm cells, except for a small cylindrical region occupied by the embryo.

Development of the embryo begins with an elongation of the zygote (fig. 16). The first division is transverse. The daughter cell next to the micropyle is elongate, the other is small and rounded (fig. 17). Each of these cells divides transversely, giving rise to four cells that are always arranged in a linear series in *Daucus* (fig. 18).

The arrangement of cells in the 4-celled embryo and the way in which each of these takes part in the formation of the mature embryo are matters which SOUÈGES (**13, 14**) regards as of great importance in the embryogeny of angiosperms. He shows, as a result of studies of representatives of many families, that 4-celled embryos may be separated into two main types on the basis of the cellular arrangement. One type has the linear arrangement just described for *Daucus*, and is characteristic of such families as the Solanaceae and Rubiaceae. The other type has the two distal cells of the four side by side, making the embryo three cells long. This type, which is the more common of the two, is found in the Compositae, Ranunculaceae, Liliaceae, Cruciferae, and other families. In nearly all cases, all members of a family produce either one or the other of these 4-celled types. SOUÈGES has also carefully determined, for species both in the same and in different families, the exact parts of the mature embryo that arise from each cell of the 4-celled embryo. From these observations (**13, 14**) he draws two conclusions: (1) each cell of a

4-celled embryo gives rise to a definite region in the mature embryo which is frequently the same in all members of the family or larger group, but differs from one group to another; (2) the way in which



FIGS. 15–27.*—Fig. 15, *a-e*, diagrams showing usual course of development of *Daucus* embryo to 16-celled stage. Figs. 16–19, longitudinal sections of 1-, 2-, 4-, and 8-celled embryos. Figs. 20–22, longitudinal sections of embryos in about 16-celled stage and older. Fig. 23, *a-f*, successive cross-sections of embryo of approximately same stage of development as that of fig. 21. Fig. 24, longitudinal section of embryo somewhat older than that of fig. 22; suspensor not shown. Fig. 25, transverse section through embryo in about same stage of development as that of fig. 24. Fig. 26, longitudinal section of embryo with dermatogen, periblem, and plerome initials differentiated; suspensor not shown. Fig. 27, cross-section of embryo in approximately same stage of development as that of fig. 26; ×750.

* See page 37 for explanation of symbols used in figs. 15–31.

the derivatives of each cell of the four enter into the formation of the parts of the mature embryo constitutes a character which is of use in the determination of plant relationships.

Since it will be necessary to refer repeatedly to specific cells of the 4-celled embryo and their derivatives, they will be designated as follows: The cell next to the micropyle will be termed the *proximal* cell (P); the cell next below it will be termed the *infra-median* cell (I); the cell next below this will be referred to as the *supra-median* cell (S); while the cell farthest from the micropyle will be called the *distal* cell (D) (figs. 15 *c*, 18). In *Daucus* all cells of the 4-celled embryo nearly always divide transversely. The cells of the filamentous 8-celled embryo thus formed are designated throughout this paper in the following manner. The two derivatives of the proximal cell are referred to as P_1 and P_2, those of the infra-median cell as I_1 and I_2, those of the supra-median cell as S_1 and S_2, and those of the distal cell as D_1 and D_2 (figs. 15 *d*, 19). In all cases the subscript 1 refers to the proximal of two sister cells and the subscript 2 to the distal of the two. The few 8-celled embryos which arise in a manner different from that just described will be discussed later.

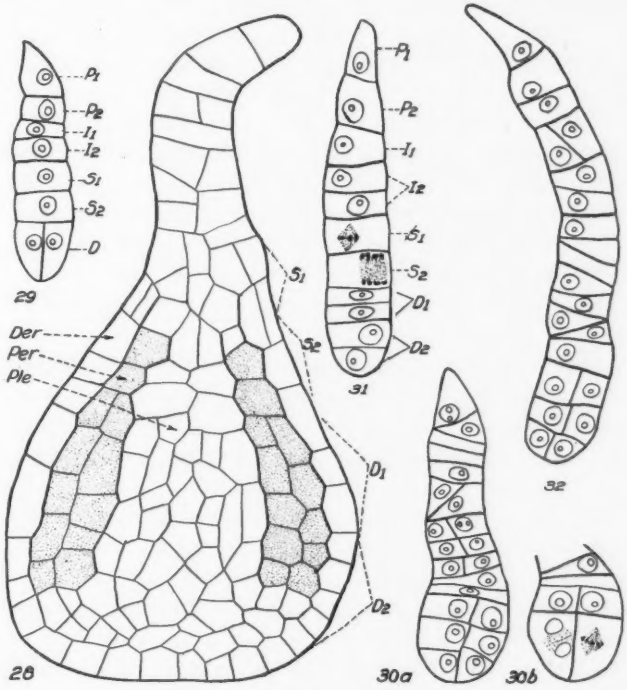
From the 8-celled stage onward the planes of division are less regular in orientation, and consequently more than one method of development may occur. The following account, based on the study of a great number of embryos, describes what is considered the most frequent course of development after the 8-celled stage. The three cells farthest from the micropyle (D_2, D_1, S_2) divide longitudinally; the other cells usually divide transversely. If these divisions were always in the planes just described, the result would always be a 16-celled embryo, 13 cells long (fig. 15 *e*). Such is seldom the case, however, since the plane of division in any of the five cells nearest the micropyle may be vertical or diagonal (fig. 20). In such cases the length of the embryo is correspondingly shorter. Moreover, the three cells farthest from the micropyle (D_2, D_1, S_2) may redivide before all of the other five have completed their first division, in which event the 16-celled stage of fig. 15 *e* never occurs. For example, in fig. 20 one of the cells usually dividing transversely (S_1) has divided diagonally, and another (I_1) is dividing vertically. Another example is shown in fig. 21, where the fifth cell (S_1) from

the micropyle has divided vertically. It will be noted that in these embryos there is a vertical division in the three distal cells of the 8-celled stage. The differences that arise are caused by the irregularity of direction or sequence of division in the five cells nearest the micropyle.

Before considering the various other types of development found in young embryos of *Daucus*, it may be well to follow the development of this first type through to maturity of the embryo. Interpreting the later stages on the basis of the 8-celled embryo, we find that the derivatives of the three distal cells (S_2, D_1, D_2) again divide vertically. As a result three tiers of four cells each are formed (figs. 21; 22; 23 *e*, *f*). The next division of each of these twelve cells is usually periclinal, forming an inner and an outer cell (fig. 24), but occasionally an anticlinal division occurs first, followed by a periclinal one in one or both derivatives, as in the lower quadrant of fig. 25. The outer cell formed by the periclinal division gives rise to the dermatogen in which further divisions are always anticlinal. The inner cell divides by a periclinal division into an outer cell which gives rise to the periblem and an inner cell that forms the plerome (figs. 26, 27).

Returning to the proximal derivative of the supra-median cell (S_1), this frequently divides transversely, but it may divide diagonally (fig. 20) or vertically (figs. 21, 22). In any case it forms a plate of four cells which serves to complete the root tip (figs. 21, 26). Differentiation of the root cap takes place comparatively late, as can be seen in fig. 28, where periclinal divisions of a few dermatogen cells give the only evidence of its formation even in this stage. While the four distal cells of the 8-celled embryo have been undergoing the development just described, the other cells continue to divide in various planes to produce a massive and usually somewhat bent suspensor (fig. 28). A mature embryo, therefore, stands in the following relationship to the cells of the 4-celled stage. The distal cell (D) of the 4-celled embryo gives rise to the cotyledons and the upper part of the hypocotyl; the supra-median cell (S) gives rise to the lower part of the hypocotyl, the root tip, and the upper part of the suspensor; the infra-median and proximal cells (P, I) are concerned only with formation of the massive suspensor.

It has already been mentioned that there are occasional exceptions to the linear condition of the 8-celled stage. One such case is shown in fig. 29, in which the distal cell (D) of the 4-celled stage has divided



FIGS. 28–32.—Fig. 28, longitudinal section of older embryo showing parts derived from each of four distal tiers of 8-celled stage: *Der*, dermatogen; *Per*, periblem; *Ple*, plerome. Fig. 29, longitudinal section of 8-celled embryo derived from 4-celled one in which distal cell divided vertically. Fig. 30, *a*, *b*, two successive longitudinal sections of embryo derived from one similar to that of fig. 29. Fig. 31, longitudinal section of embryo derived from 8-celled type in which three distal cells have divided transversely. Fig. 32, older stage of embryo derived from one similar to that of fig. 31; ×750.

longitudinally. Although this was the only embryo of this type observed in a great number of 8-celled ones examined, older stages were found which had obviously developed from an 8-celled embryo of similar constitution. For example, in fig. 30, which shows two con-

secutive sections of the same embryo, it is evident that the distal cell (D) of the 4-celled stage first divided longitudinally, and its two daughter cells also divided longitudinally. These four cells then divided transversely to form two layers of four cells each, thus making the end result the same as would have occurred had the distal cell divided transversely in the first place. Evidence for this sequence of divisions is found in the direction of the mitotic figures in one side of the embryo, in the failure of the transverse walls to coincide in the other side, and in the continuity of the longitudinal wall in each section through both distal layers of cells (fig. 30). A study, by means of these criteria, of a great number of 8-celled and more advanced embryos shows that longitudinal division of the distal cell (D) of the 4-celled stage is relatively uncommon. Development subsequent to this stage is the same in this type as in the type described previously.

Although the three distal cells (D_2, D_1, S_2) of a linear 8-celled embryo usually divide longitudinally (fig. 15 *d*), such is not always the case. For example, in fig. 31 there are already 11 cells formed in single file, and two of these are undergoing transverse division, so the embryo will be at least 13 cells long before any longitudinal walls can be formed. This embryo was derived from a linear 8-celled one by transverse division of each of the five cells farthest from the micropyle. The three proximal cells of the eight are still undivided. A somewhat older stage of apparently a similar origin is shown in fig. 32. It seems probable that in this case also, one or more of the three distal cells of the 8-celled embryo underwent transverse division before longitudinal walls were formed. It is to be noted, however, that when longitudinal walls were formed they occurred in the three distal cells only, indicating that the mature embryo will be formed in the usual way from three layers of cells. It seems probable that the three distal cells of fig. 31 will next undergo longitudinal division, and will probably give rise to all of the embryo except the root tip. If this occurs, the embryo will be derived entirely from the distal cell of the 4-celled stage instead of from the distal and supramedian cells, as more frequently happens. Before discussing the significance of embryos of this type it will be well to consider development of the embryo of *Carum* as described by SOUÈGES (**17**).

In *Carum carvi*, SOUÈGES reports the regular formation of a linear 4-celled embryo similar to that always found in *Daucus*, but finds three different types of 8-celled embryos arising from the 4-celled one. One of these types results from transverse division of each cell of the 4-celled stage, and is similar to the 8-celled embryo usually found in *Daucus*. Another 8-celled type has a longitudinal division of the distal cell and a transverse division of the other three. It is similar, therefore, to the aberrant type of *Daucus* embryo shown in fig. 29. The third type in *Carum* has the distal and supra-median

TABLE I

ORIGIN OF PARTS OF MATURE EMBRYOS OF CARUM AND DAUCUS FROM CELLS OF 4-CELLED EMBRYO

CELL OF 4-CELLED EMBRYO	PART OF MATURE EMBRYO DERIVED FROM EACH CELL OF 4-CELLED STAGE		
	Carum	Daucus	
		Usual type	Aberrant type
Proximal	Suspensor	Suspensor	Suspensor
Infra-median	Part of suspensor; root tip	Suspensor	Suspensor
Supra-median	Hypocotyl	Part of suspensor; root tip; lower part of hypocotyl	Suspensor
Distal	Cotyledons	Upper part of hypocotyl; cotyledons	Root tip; hypocotyl; cotyledons

cells dividing longitudinally and the other two dividing transversely. No embryo of this type has been found in *Daucus*. SOUÈGES studied the subsequent development of each of these types in *Carum*, and concluded that in all three cases the cotyledons are derived from the distal cell of the 4-celled embryo, the hypocotyl from the supra-median cell, the root cap and part of the suspensor from the infra-median, and the rest of the suspensor from the proximal cell. If this is true, there are some striking differences between *Daucus* and *Carum*. These differences, together with the differences that occur between different individuals of the same species of *Daucus*, are summarized in table I.

It will be recalled that SOUÈGES places much emphasis upon both the arrangement and destiny of the cells in the 4-celled embryo. He states that in *Carum* these embryos are always linear. My observations indicate that the same situation holds in *Daucus*. The studies of HEGELMAIER (**8**), HÅKANSSON (**7**), and TANFANI (**19**) show that in a number of other genera of the Umbelliferae linear 4-celled embryos are regularly produced. The other conclusion of SOUÈGES, that within the family the destinies of the cells of the 4-celled stage are usually the same, is not supported by this study of *Daucus*, as shown in table I. This table shows that the parts of the mature embryo of *Carum* do not originate from the same cells of the 4-celled embryo as do those of *Daucus*. SOUÈGES' figures show that there is a possibility that he has misinterpreted the method of origin of certain of the advanced stages. If this proves to be the case, the development of *Carum* will be found to be the same as that found most frequently in *Daucus*. The table also shows, however, that each cell of the 4-celled *Daucus* embryo does not always give rise to the same part of the mature embryo. It is of interest to note that these aberrant types are of such a nature that they increase rather than decrease the differences between *Daucus* and *Carum*. The conclusion of SOUÈGES, that the destiny of the cells of the 4-celled embryo is rather constant throughout a family, obviously does not hold in the Umbelliferae, because in this family we find differences not only between different genera but between individuals of the same species. Until more genera have been investigated it will be unsafe to attempt any generalization as to the manner in which the cells of the 4-celled stage contribute to the mature embryo in the Umbelliferae.

Summary

1. This paper is concerned with the development of the female gametophyte, the pollen tubes, and the embryo of *Daucus carota*.

2. A single archesporial cell functions directly as a macrospore mother cell which produces a linear tetrad of macrospores.

3. The macrogametophyte, which arises from the chalazal macrospore, is of the 7-celled type characteristic of most angiosperms.

4. The presence of a cellulose filiform apparatus was demonstrated by microchemical tests.

5. The structure and path of the pollen tubes were investigated. Tubes were found to grow intercellularly down through the conducting tissue of the style to its base, and then superficially along a groove leading to a canal communicating with each locule. Tubes growing down one style may enter the locule immediately below or grow through the transverse canal and into the other locule.

6. A filamentous 8-celled embryo is usually formed before longitudinal divisions occur. The three cells farthest from the micropyle give rise to all of the embryo except the root tip. The other five cells give rise to the root tip and the suspensor.

7. Development of the embryo was considered in the light of SOUÈGES' theory that the 4-celled embryo is the critical stage in development, and that for any given species the four cells always give rise to the same parts of the embryo. In *Daucus* the mature embryo may arise entirely from the distal cell of the 4-celled embryo, or from the distal cell and derivatives of the cell next to it. SOUÈGES' further generalization that embryonal development is usually characteristic throughout a family does not appear to hold in the Umbelliferae, as is shown by a comparison of *Daucus* with his account of the embryogeny of *Carum*.

The author takes this opportunity to thank Dr. GILBERT M. SMITH for his assistance during the preparation of this paper.

COLLEGE OF AGRICULTURE
UNIVERSITY OF CALIFORNIA
DAVIS, CALIF.

[*Accepted for publication August 13, 1930*]

LITERATURE CITED

1. ARTSCHWAGER, ERNST, Use of chloroiodide of zinc in plant histology. BOT. GAZ. **71**:400. 1921.
2. BEGHTEL, F. E., The embryogeny of *Pastinaca sativa*. Amer. Jour. Bot. **12**: 327–337. 1925.
3. BRADBURY, DOROTHY, A comparative study of the developing and aborting fruits of *Prunus cerasus*. Amer. Jour. Bot. **16**:525–542. 1929.
4. CAMMERLOHER, H., Studien über die Samenanlagen der Umbelliferen und Araliaceen. Österr. Bot. Ztschr. **60**:289–300; 356–360. 1910.
5. DAHLGREN, K. V. OSSIAN, Die Befruchtungserscheinungen der Angiospermen. Hereditas **10**:169–229. 1927.

6. HABERMANN, A., Der Fadenapparat in den Synergiden der Angiospermen. Beih. Bot. Centralbl. **20**:300–317. 1906.
7. HÅKANSSON, A., Studien über die Entwicklungsgeschichte der Umbelliferen. Lunds Univ. Arsskr. **7**:1–118. 1922.
8. HEGELMAIER, F., Vergleichende Untersuchungen über die Entwicklung dikotyledoner Keime mit Berücksichtigung der Pseudo-monokotyledonen. Stuttgart. 1878. Cited by HÅKANSSON.
9. JURICA, H. S., A morphological study of the Umbelliferae. BOT. GAZ. **74**: 292–307. 1922.
10. LEE, B., The microtomist's vade-mecum. 9th ed. Philadelphia. 1928.
11. MANGIN, L., Sur la callose, nouvelle substance fondamentale existant dans la membrane. Compt. Rend. Acad. Sci. Paris **110**:644–647. 1890.
12. MEZ, C., Beiträge zur Kenntnis des Umbelliferen-Embryos. Verh. Bot. Vereins Prov. Brandenburg **29**:30–36. 1887.
13. SOUÈGES, R., Embryogénie des Liliacées. Développement de l'embryon chez l'*Anthericum ramosum*. Compt. Rend. Acad. Sci. Paris **167**:34–36. 1918.
14. ———, Les premières divisions de l'oeuf et les différenciations du suspenseur chez le *Capsella bursa-pastoris* Moench. Ann. Sci. Nat. 10. Bot. **1**:1–28. 1919.
15. ———, Recherches sur l'embryogénie des Solanacées. Bull. Soc. Bot. France **69**:163–178; 236–241; 352–365; 555–585. 1922.
16. ———, Embryogénie des Rubiacées. Développement de l'embryon chez le *Sherardia arvensis* L. Compt. Rend. Acad. Sci. Paris **178**:1919–1921. 1924.
17. ———, Embryogénie Végétale. Embryogénie des Ombellifères. Développement de l'embryon chez le *Carum carvi* L. Compt. Rend. Acad. Sci. Paris **182**:339–341. 1926.
18. STRASBURGER, E., Über Befruchtung und Zellteilung. Jenaische Zeitschr. **11**:435–536. 1877.
19. TANFANI, E., Nota preliminare sul frutto e sul seme delle Apiaceae. Nuovo Giorn. Bot. Ital. **20**:307–313. 1888.
20. TSVETT, M. S., Sur un nouveau réactif colorant de la callose. Compt. Rend. Acad. Sci. Paris **153**:503–505. 1911.

UNUSUAL ASPECTS OF MEIOTIC AND POSTMEIOTIC CHROMOSOMES OF GASTERIA[1]

HSU-CHUAN TUAN[2]

(WITH PLATES I, II AND FOURTEEN FIGURES)

Introduction

The investigation here described was initiated at the University of Pennsylvania in 1929. The preliminary work consisted of a study of the comparative action of different fixing reagents and of the methods of applying different nuclear stains. The investigation covered a wide range of plant material.

The technique of making cytological smears (**20**) rendered the question of fixation comparatively simple. Emphasis was therefore placed upon the acquiring of a satisfactory staining technique. It was found that picric acid destains material which has previously been treated with haematoxylin and safranin, and by its use the writer was able to get consistently satisfactory results. *Gasteria* was first investigated, and subsequently smears of *Clarkia*, *Solanum*, *Pinus*, and many others were studied experimentally. In the course of this study, a group of *Gasteria* smears, showing microsporogenesis, yielded an unusual phase of diakinesis chromosomes. This discovery led to the present report. In diakinesis, some cells showed a sudden change in their capacity for absorbing the stains, and in addition they were found to go through a regressive process to a false resting stage. Often the pollen mother cells of this particular plant divide three successive times, forming eight pollen grains.

TAYLOR (**21, 23, 26**) had previously made a thorough study of *Gasteria* chromosomes, and contributed much to our knowledge of their shape and structure. A comparable investigation of this series of abnormal chromosomes in the same genus might therefore be expected to reveal other interesting facts.

[1] Contribution from the botanical laboratory of the University of Pennsylvania.

[2] Fellow of the Chinese Educational Mission at Washington, D.C., of Tsing Hua University, Peiping, China.

A part of this work was done at the Marine Biological Laboratory, Woods Hole, in the summer of 1930, after the material had been collected and partially studied at the University of Pennsylvania. The research was then completed at the botanical laboratory of the University of Michigan. To these institutions the writer is much indebted for laboratory space and the necessary equipment.

Material and method

Pollen mother cells of *Gasteria* were collected in a greenhouse at the University of Pennsylvania in the spring of 1930. Young buds were teased out with a needle and the anthers transferred immediately to a thoroughly cleansed slide. With a stroke of a scalpel, the pollen mother cells were smeared evenly into a thin film on the slide, which was then submerged in the fixing fluid for 20 minutes. Many fixing fluids were tried, and although the results varied much in detail, the fixation of smears was generally better than preparations sectioned by the paraffin method. The following account is based upon the result obtained by the use of a special chromic-acetic-osmic fixative developed by TAYLOR (MCCLUNG 11) for *Gasteria* pollen mother cells. No paraffin preparations were used, since the smears give the most natural image of the cell, both in regard to its general contour and to the details of its chromosomes. Shrinkage is almost eliminated in most of the cells. The staining technique described by TUAN (**27, 28**) was followed, with some slight modification to suit each individual case. When haematoxylin was used, the picric acid differentiation was occasionally abbreviated by the slides being placed in a 0.5 per cent iron alum solution for 2 minutes before they were transferred to the picric acid for further differentiation. This saves the long schedule of differentiation required when picric acid alone is used. For *Gasteria*, however, the writer still prefers the long schedule of picric acid, but in the case of *Tradescantia*, prepared for purposes of comparison, a previous treatment in a 0.5 per cent iron alum solution cut down the time from 3 hours to 30 minutes. When stained with safranin, the slide was brought into a mixture of two parts of clove oil and one part of 95 per cent alcohol, and there the differentiation was further adjusted. Deeply stained or over stained slides can be saved by this process.[3] One example

[3] This alcohol-clove oil differentiation can also be applied to crystal violet smears.

from each of the methods illustrates their general application to the *Gasteria* study:

	Minutes
Slide PK 8, Safranin	
1. Stained in Grubler's safranin O	5
2. Picric acid-alcohols	5
3. Ammonia-95% alcohol	0.5
4. Pure 95% alcohol	0.5
5. 95% alcohol-clove oil mixture	5–10
Slide KP 9, Haematoxylin[4]	
1. 2% iron alum	20
2. ½% haematoxylin	5
3. Differentiated in picric acid	160
Slide KP 6, Haematoxylin	
1. 2% iron alum	20
2. ½% haematoxylin	5
3. ½% iron alum	2
4. Differentiated in picric acid	30

Investigation

In the normal behavior of *Gasteria* chromosomes, according to Taylor (**21, 23, 26**), the first suggestion of prophase activity in meiosis is marked by an aggregation of chromatic granules, forming more or less spiral chromonemata. When the spirals straighten out from a tangled condition to form the extended chromatic threads, synapsis of homologous chromosome pairs occurs simultaneously at different places throughout the nucleus. The longitudinal split of the paired chromosomes, which form the tetrad, is accomplished before the diakinesis stage. Three pairs of pyriform chromosomes and four pairs of larger chromosomes are present on the metaphase plate. The tetrad condition of each pair is revealed distinctly. A coiled spiral of from seven to nine turns is present in the longer chromosomes, while in the small ones the number of coils is correspondingly reduced. These coils apparently are a result of the contraction of the more or less straight chromonemata of an earlier stage. In the anaphase, the two chromatids of each dyad may separate and the coils within them become more distinct. When telophase is reached, the chromosomal sheath is no longer stainable but

[4] When haematoxylin is used, each stage is followed by a prolonged washing in tap water.

is still present. Eventually the chromosomes become disorganized in the interphase. When they reappear for the second division, their shape and internal structure acquire interest from a new angle. At the proximal end of each chromosome a mild constriction is invariably present; perhaps in the three short ones a secondary constriction occurs along the shaft. In size, the homeotypic chromosomes are more slender and longer than the heterotypic ones. The internal structure of each of the homeotypic chromosomes resembles that of the somatic chromosomes, that is, each chromosome contains two chromonemata derived from a split of the coils. A quartet is formed as a result of two cell divisions. Each of the resulting cells divides again after the formation of a thick wall, typical of pollen grains.

There are several striking differences between the normal behavior of *Gasteria* chromosomes, as just described, and the peculiar mode of division to be recorded in the next few paragraphs.

The first sign of abnormality is a dispersal of the diakinesis chromosomes to form a false resting stage. More complicated abnormalities occur after the first metaphase. The nuclear membrane fails to form at telophase. Two sets of second metaphase chromosomes persist through the omission of the interphase and second prophase. The chromosomes thus produced assume two different aspects. The type (fig. 30) with distinct spiral structure is identical with that which appeared in the first metaphase; the other type (figs. 2, 8, 10, 18) is erratic and cannot be connected with any particular division of the chromosomes. So far as their outer form is concerned, they are intermediate between the homeotypic and heterotypic chromosomes, as they have extraordinary terminal heads and short, heavy bodies. Abnormal chromosomes of another type (fig. 9) are produced by some cells in which a nuclear membrane is formed after the telophase. The probable fate of these last was not determined, owing to a lack of sufficient material of this type, but the history of the chromosomes with spiral structure is traced to the formation of third division chromosomes within the nucleus of quartet cells (fig. 7). No regular division of the quartet was observed, but furrows of various depths occur in these cells, and have been noted in many preparations. This suggests that the third division is carried through by a simple process of furrowing. On the

FIGS. 1–14.—Fig. 1, selected chromosome sets showing four types of chromosomes: *A*, first metaphase chromosomes; *B*, second metaphase chromosomes with extraordinary heads; *C*, second metaphase chromosomes with shape and appearance of first metaphase chromosomes retained; *D*, set of constricted chromosomes. Fig. 2, second metaphase figure showing giant headed chromosomes scattered irregularly in cytoplasm; 28 elements counted in all, but only 26 shown. Fig. 3, pair of anaphase chromosomes showing bulging matrix. Fig. 4, early telophase showing partial rotation of daughter groups. Fig. 5, one of constricted chromosomes from binucleate cell showing satellite at lower end. Fig. 6, mid-anaphase figure showing partial rotation of daughter groups and non-separation of single chromatid. Fig. 7, third division chromosomes with all types of constrictions represented. Fig. 8, group of second metaphase chromosomes showing giant headed chromosomes and extruded dyad. Fig. 9, constricted chromosomes from binucleate cell. Fig. 10, two sets of giant headed chromosomes showing extruded dyad at periphery. Fig. 11, extrusion of small chromosome at equatorial plate stage. Fig. 12, 8-celled polyspore. Fig. 13, second metaphase figure showing extrusion of dyad from each of two sets of chromosomes. Fig. 14, 9-celled polyspore.

other hand, these chromosomes, like some of the diakinesis elements, may disintegrate and produce false resting nuclei.

Regression of chromosomes from diakinesis

The clearest evidence of tetrad chromosomes was observed during the diakinesis stage (fig. 16). From the general appearance of these threads, it seemed that the cell was perfectly normal, and had behaved as described by Taylor. The only peculiarity up to the first metaphase was a suggestion of disintegration of the thickened diakinetic chromosomes, which were recognized in a few cells by the fact that they stained faintly (fig. 17). When these chromosomes are traced through several stages, it is found that they collapse completely. The tetrad is gradually brought clearly into view with the homologous chromatids connected by numerous achromatic bridges (fig. 32). The chromosomes may be compared to a rope ladder. In a later stage the achromatic bridges are replaced by a clear space, so that parallel threads of an early prophase nature are formed (fig. 29). Further disintegration of the threads brings about a false resting stage, which might be mistaken for the earliest evidence of the first prophase, except that the nucleolus in this case is absent. Probably cells of this type will not divide again, as there was no evidence to show a reorganization for further division.

Those cells which show the same kind of disintegration but at a later stage will not be described in detail. A brief account of their development, however, may bring out some points for discussion. (1) Empty cells are frequently observed among all stages of meiotic figures. (2) Cells at the binucleate stage or quartet stage which show only a large but faintly stained nucleus are also frequent. (3) Nuclei of a quartet may show different stages of activity (fig. 40), sometimes from one to three nuclei of a quartet being missing. Whether these cells represent a complete breakdown of the chromatin material or not could not be determined, but from this example of the diakinetic chromosomes it seems likely. As to the cause of these abnormalities, it is rather rash to connect them with any one agent, such as pathological influence or genetical unbalance. It is most probable that there were other forces which, in addition to these, contributed much to the peculiar behavior of these cells.

FIRST METAPHASE.—The lapse of time from diakinesis to metaphase is exceedingly short. This observation is confirmed by a general lack of intermediate stages in many preparations, although in a few cases figures of such were not hard to identify. When diakinesis ends, the chromosomes shorten and thicken with surprising rapidity. The scattered chromosomes are suddenly gathered up in the central portion of the cell, while the nuclear membrane disappears entirely and the fiber attachments are formed on each of the chromosomes. It seems that the chromosomes which are now not in contact with the cytoplasm are able to take up the stains more readily than at any other stage; therefore the metaphase chromosomes arranged on the equator have the appearance of solid rods, and their internal structure is detected only in well differentiated preparations. On the metaphase plate, seven pairs of chromosomes, split into tetrads (fig. 27), can be observed so far as the general contour of the cell and the chromosomes are concerned. There are, however, several striking cases of abnormal distribution. In a few cells one of the small chromosomes is found at the polar region when the rest of the set is still on the equator (fig. 11). Also, one of the small chromosomes is often extruded entirely from the spindle region (fig. 6), so that when migration is completed, it is left at the peripheral region of the equator where it rounds up quickly to form a small nucleus.

FIRST ANAPHASE.—The anaphase chromosomes can clearly be counted with seven dyad chromosomes in each daughter set. At the beginning of migration, the chromatids are evidently subjected to a strong pull between the spindle fibers and the surfaces of contact with their corresponding homologues. When they are midway between the pole and the equator, they are stretched to a certain extent, the turns of the spiral bulging out at places and suggesting the presence of an elastic matrix (fig. 3). Slender threads may be seen between them as the daughter chromatids are drawn farther and farther apart. As a rule, the partners of each dyad are separated before they reach the pole (fig. 31). In one extreme case (fig. 6) one of the chromatids was pulled across the equator and failed to separate. The telophase (fig. 4) follows, with a general lack of the compactness of the chromosome such as is noticed in the normal condition. The small chromosomes reach the pole ahead of the large

ones, although migration started simultaneously in all. Many cells now pass quickly to a second division without any of the phenomena associated with the heterotypic telophase. Only the least abnormal types, those which retain some evidence of telophase dispersal, will next be considered. However, a brief partial telophase may result in the formation of chromosomes as seen in figs. 2, 8, 10, and this will be described in connection with the second metaphase chromosomes.

Residual first telophase and derived chromosomes

The ends of the chromosomes toward the polar region approach each other, but the distal ends, extending toward the equator, alter their position very little. This is soon followed by a gradual rotation of the two groups of chromosomes in opposite directions (figs. 4, 6). After a complete turn of 90° they lose their staining ability. There are a few examples of nuclear membrane formation, but the majority of the cells do not show such a tendency. The former type of nuclei lead to another abnormal division, while the latter (fig. 24) soon recover their staining properties, so that they are seen as full sized chromosomes on the second metaphase plate (fig. 30). The interphase and second prophase of the latter type of nuclear division are entirely skipped, and consequently the appearance and structure of the chromosomes are unchanged from the first division, a condition similar to that of some animals in which the interphase and prophase are skipped during maturation divisions of the egg nucleus after entrance of the sperm.

In those cells in which the nuclear membrane plays a part, the chromosomes show a progressive loss of staining ability. The membrane appears first at one side, usually the side near the polar region (fig. 21), whence it grows about the mass to complete its formation (fig. 19). At this stage, as the nucleus increases in size, the chromatids are so lengthened that a continuous but loosely coiled spiral is seen in each (figs. 19, 21). Further stretching of the spirals (fig. 20 *a*, *b*, *c*) gives rise to an isolation of a single loop at the proximal end of each chromosome. Later the nuclear membrane, perhaps as a result of the excess lengthening of the chromosomes, is ill-defined and suggests that it is in the process of disintegration. Further evidence derived from the formation of heavily constricted

chromosomes (fig. 9) would seem to indicate that the nuclear membrane is finally and entirely disorganized. The cause of such a phenomenon was not determined, but it is interesting to note that immediately after disappearance of the membrane, two sets of constricted chromosomes are found in the cell. The origin of this type of chromosomes is probably that as soon as isolation of the tips of the spirals is accomplished (figs. 20, 23), anastomoses appear (figs. 37, 38). At first they are difficult to recognize, but later they assume a distinct threadlike form (fig. 9). This process is accompanied by the vacuolation characteristic of telophase chromosomes. The nuclear membrane is indefinite, and its complete collapse initiates reorganization of the chromosomes. A count of the elements in each of the cells reveals fourteen as the number (fig. 23). Two of them bear extremely big heads, which are further divided by another contraction (fig. 9). Six of them show comparatively small heads, while each of the six small chromosomes is terminated by a round head and has also a median constriction. All of these heads can be mistaken for satellites, as they are connected to the shafts by very slender threads. In one case, one of the large chromosomes showed a small satellite at the distal end (fig. 5). Presence of true satellites in meiotic chromosomes has not yet been reported, and their purely vegetative nature was emphasized by TAYLOR (**23**). No inference can be drawn from this sole exception.

SECOND METAPHASE.—It has already been mentioned that the interphase and prophase are skipped in many of the cells, owing to failure of the nuclear membrane to appear. This account of the behavior of these cells will therefore begin with the metaphase of the second division.

The chromosomes which retain the form they had in first division telophase have now recovered their staining ability in a surprising manner (fig. 24), each of the two sets of metaphase chromosomes being arranged on a second equator for the following division (fig. 30). The plane of this division is perpendicular to that of the first, as is shown by the rotation of the daughter groups in the earlier stages. From these they can clearly be seen as two sets, not arranged in a definite pattern. No case could be found in which the chromosomes were not widely separated from one another; this is

perhaps one of the reasons that a lagging and an extrusion of chromosomes will later occur. Rejection of a dyad chromosome was frequent, while the elimination of a dyad or two (fig. 13) from each daughter set of chromosomes was common. Those eliminated may give rise to some supernumerary spores.

Another type of abnormality is found at the second metaphase plate. Although many chromosomes show an internal spiral structure, there are some cells whose chromosomes show a striking difference (figs. 2, 8, 10, 18). These latter are constricted, with extraordinary heads at the tip, while the body is of a peculiar type, short and heavy. By an analysis of all the figures available, the origin of these chromosomes was traced back to the first telophase, when the nuclear membrane was just about to form. Consequently it is most probable that at telophase the nuclear membrane of these cells has a very brief existence, so that the chromosomes are only partially disorganized for the homeotypic division. Further collapse of the spindle would undoubtedly give rise to these scattered chromosomes (fig. 2). In fig. 2 the total count of the chromosomes is 28, but only 26 can be shown in the drawing. The count of 28 is exactly four times the haploid number. From the size of the heads, it is further determined that the total complex represents four sets of chromosomes, and each set (fig. 1 *b*) has the haploid number of seven. For these chromosomes at least, it shows that the chromatids of each tetrad are completely separated at late anaphase. Identified by the heads, these chromosomes are traced to the resting quartet stage. Late anaphase cells are frequently seen with four sets of chromosomes spread widely in the cytoplasm (fig. 26). Disintegration of the chromosomes, typical of telophase activity, occurs. Formation of a nuclear membrane is postponed until disintegration of the chromosomes is almost completed (fig. 33 *a–e*).

SECOND ANAPHASE.—The second early anaphase is brief. Cells are often observed in late anaphase (fig. 25) and telophase. It has been stated that the metaphase chromosomes are loosely scattered along the equatorial region, therefore it is to be expected that various abnormalities of the chromosomes would appear in the second anaphase. Several cases of chromosome extrusion from the spindle regions have been mentioned in connection with the metaphase chro-

mosomes, but as the appearance and history are similar to what has been described, a repetition will not be attempted.

Second telophase and third division chromosomes

In many cells, when the chromosomes have reached their respective poles, they round up to form the telophase. Ultimately a regular resting quartet is formed, all the chromosomes having lost their individuality within the nuclear membrane. Probably the meiotic activity of the cells developing in this manner is thereby concluded. There are a number of quartets, however, which retain the spirals throughout the telophase in perfect shape until a new set of active chromosomes is formed (fig. 7). Although these chromosomes were not observed to go through an elaborate division, their formation in the quartet nucleus is sufficient evidence for such a possibility. The history of the spirals here seems to be of the same nature as that shown in the first telophase, although the number in this case is reduced one-half. Furthermore, the constricted chromosomes formed after second telophase (fig. 9) are identical with those found after the first telophase. The development of these chromosomes could be only partially traced. It may be said, however, that at the end of the second telophase, the chromosomes gradually lost their chromaticity as the coiled spiral reappeared (figs. 28, 39). Each of the spirals then elongates at the expense of its width, so that the coils become very narrow compared with those first involved in this process. A loop or two at the tip of each spiral is then gradually dragged apart from the main body as the chromosomes reach their maximum length. The chromosome number is seven: one bears a big head (fig. 1 *d*), three are terminated by smaller heads, while at the tip of the small chromosomes a round head is shown with a secondary constriction along the shaft. This combination is exactly like that found after the first telophase, except that each type is represented by a single element. There is not much detail to be seen in these chromosomes. Their structure was discerned, however, in other cells in which the chromosomes were undergoing a process of regression. They had a single spiral. These chromosomes resemble the homeotypic ones in their constrictions, but they are different in structure, as the homeotypic chromosomes under normal condi-

tions have two spirals in each. The question arises as to whether or not this type of abnormality should be connected with the normal homeotypic chromosomes. The writer will develop this point later.

Several interesting figures may be observed in the regression quartets. The quartet nuclei in many cases show different stages of activity in the same group (fig. 40 *a-c*). A typical resting nucleus is shown in *b; c* is shown with partially disintegrated chromosomes; while *a* is intermediate. From the equal sized and equally stained nucleoli in these cells, it is probable that these stages are passed through in a short period, and the probable disturbance occurs immediately after formation of the third division chromosomes. Reformation of the nuclear membrane later will mark the beginning of the regression process. The most probable steps of chromosome behavior in these cells are as shown in fig. 22 *d*, *c*, *b*, *a*, and figs. 36, 40. The earliest stage of disintegration is marked by a loss of chromaticity of the chromosome matrix, so that the comparatively heavily stained spiral within the chromosomes is brought out in better contrast (fig. 22 *c*). The chromosome at the lower right hand corner of fig. 36, and two other segments in the same cell, show the spiral clearly, while the farther ends of these chromosomes are in a more advanced stage of disintegration. Increasing number of anastomoses and central vacuolation (fig. 22 *a*, *b*) bring the chromosomes close to a resting stage (fig. 40 *b*). It seems that the chromaticity of each spiral varies at regular intervals, and usually certain segments are still heavily stainable while others register little affinity for the stain (fig. 40 *a*), so nodules are distinctly shown at places throughout the nucleus. When vacuolization reaches a climax, the chromosomes appear double, with faintly stained peripheral regions and clear central cylinder (fig. 40 *b*). Besides these regression figures, there are two selected from a number of quartet cells worth mentioning. The one (fig. 34) with short spirals is rather difficult to assign to a particular developmental position. It is probably a second telophase figure, however, with the spirals segmented into short pieces. The other (fig. 35), with slender but continuous spirals, has been given no definite position either. There are four longer spirals and three shorter ones. If it is not one of the second telophase figures, it must be classified with the regression chromosomes, and should be followed closely by the spirals shown in fig. 36.

Polyspory

In several preparations, 8-celled spore groups are found among other abnormal groups containing from five to many cells in place of the normal quartet. It has been suggested that the lagging of chromosomes at first metaphase and at many other stages may produce extra nuclei, therefore all the supernumerary cells in any of these groups may be considered to be the natural consequence of such abnormalities. A detailed consideration of the 8-celled groups strengthens the assumption that third division does occur in some of the cells.

Eight nuclei of equal size frequently occur in 8-celled groups (fig. 12), while one or two small extra nuclei (fig. 14) are also frequently present. It is easy to account for the small nuclei as the result of misplaced chromosomes, but as to how the eight equal sized nuclei developed, there is only one reasonable explanation; that is, that they are the product of a third division. The evidence in support of the presence of a simple third division may be summed up in a few words. As soon as the chromosomes are formed in each of the four nuclei of a quartet, the nuclear membrane disappears, although the nucleolus, formed at the end of the telophase, is still obvious (fig. 7). The chromosomes, usually seven in number, spread apart in the cell without sign of spindle formation. The next stage, thus far observed, involves cleavage of various depths along the cell wall and cytoplasm. Whether this is the mode of the third division or not is without further proof, and the method by which these chromosomes are distributed to the daughter nuclei is another unanswered question. Additional work on the pollen mother cells of this plant may bring to light more evidence.

Rôle of nucleolus

From the resting cells up to the diakinesis stage, the nucleolus remains in the nucleus with little change. Its diameter is estimated as 2.5–3.5μ (figs. 15, 16). It disappears almost simultaneously with the nuclear membrane as the chromosomes are arranged for the first division metaphase. It is observed again as a faintly stained body at the end of the first telophase, with a much reduced diameter (1–1.5 μ). It increases in size within the nucleus containing the first type of constricted chromosomes. At this stage it measures

about 2 μ in diameter (fig. 9). With the other type of division, where the interphase and prophase are omitted, the nucleolus does not appear until the second telophase is far under way. It shows the same diameter as before; that is, it first appears as a body about 1 μ across, and then increases in bulk when the third division chromosomes are formed (fig. 7). Its definite function in this process cannot be accounted for; however, the interpretation that the nucleolus has some connection with the chromosomal appendages is eliminated. In this case it occurs simultaneously with the fully developed chromosomes, therefore it is not converted into any of these appendages.

Chromosome structure

Since the structure of chromosomes has already been discussed, only a few outstanding points will be added here. On the metaphase plate the chromosomes are heavily stained. From haematoxylin preparations, the spiral structure is not very distinct, but the number of coils was easily ascertained by the nodule-like structures which bulged out at places along the elastic matrix. It is estimated that in the longer chromosomes the spiral has from seven to nine turns, while in the small ones there are only two or three present. In many metaphase cells indication of a longitudinal split was not observed. The single nature is retained throughout until the formation of chromosomes with satellite-like appendages. As previously mentioned, this type of chromosome, in its outward appearance, resembles the normal homeotypic chromosome, but from its internal structure the resemblance is entirely lacking. The cause for such difference is probably due to the fact that the prophase, in which the split is supposed to take place, was skipped, and therefore the split was perforce omitted. The significance of this process is not understood, but it confirms the interpretation of Taylor (**26**) that the double spiral in the homeotypic chromosomes is the consequence of a longitudinal split completed at the second prophase. The internal structure of those chromosomes with giant heads is rather obscure. In many well differentiated cells, it is seen as a comparatively lighter central core with a densely stained peripheral region (fig. 18), containing well spaced nodules in two rows, a structure which makes the chromosomes appear double. When the chro-

mosomes come to the second anaphase (fig. 26) the same kind of apparent doubleness is maintained, in addition to the vacuoles and anastomoses.

Discussion and historical review

Several diverse problems are involved in the abnormal behavior of *Gasteria* chromosomes: (1) the occurrence of three successive divisions in microsporogenesis; (2) the appearance of the heterotypic type of chromosomes in the second division; (3) the exaggerated constrictions of the chromosomes; (4) the production of polyspores after the close of meiotic activity; (5) the regression of chromosomes from different stages of chromosomal development to a false resting stage; and (6) the structure of chromosomes.

Literature recording three or more divisions at the time of meiosis is scattered. Both in spore formation in Ascomycetes and oogenesis in Fucaceae, as well as in embryo sacs, it is well known that mitoses may occur after meiosis before the full number of cells is completed. The occurrence of the division at very different periods in the morphological life cycle suggests that the divisions resulting in polyspory are not unforeshadowed and altogether new in higher plants. Recently STOW (18), by applying a higher temperature to growing bulbs of *Hyacinthus*, obtained multinucleate giant pollen grains. He found that cells dividing three times in succession produce structures resembling embryo sacs, while those dividing four or five times give the same kind of irregularity but of a more complicated form. He concluded that the male potency in these cells is reduced by the physical means employed, and thus the female tendency is given a temporary dominance. Although this conclusion is hardly applicable to this case of *Gasteria*, where signs of embryo sac formation are entirely lacking, it suggests the possibility that material for this study was injured by some similar agents in the greenhouse before fixation.

As to the appearance of a similar type of chromosomes in the first and second division during meiosis, no corresponding case can be found among plants. Among certain forms of animals such a condition is of common occurrence, therefore it is rather hasty to establish any co-relation here.

Constrictions and satellites have been reported frequently as a

permanent morphological character for individual chromosomes. TAYLOR (**21, 22, 23, 24, 25**) identified certain chromosomes in many forms by the constrictions and satellites. SVESCHNIKOVA (**19**) classified the genus upon the size of chromosome heads and number of satellites seen in many species of *Vicia*. SAKAMURA (**15**) and many others, by means of the use of chloral hydrate and by following the constrictions, have settled the disputed counts in several plants and corrected the old conception of chromosomal tetrads and transverse divisions. However, the origin of such morphological characters is only partially discussed in these articles. NAWASCHIN (**12**) probably contributed the first interpretation that nucleolar material is responsible for the satellites. TAYLOR (**22**) and DARLINGTON (**3**) claimed that the satellite is the natural result of an extreme type of subterminal constriction. In the present case, it is seen that the satellite-like appendages are the direct production of isolated loops of chromatic spirals. Their coexistence with a nucleolus in many of the cells makes it obvious that the nucleolar material of *Gasteria* plays no direct part in the formation of such appendages.

There is an enormous amount of publication on polyspory. Almost without exception each article deals with hybrids or polyploid species. Taking the rose family for example, ERLANSON (**4**), in a study of American wild roses, found that polyspory is a common phenomenon among spontaneous hybrids or descendants of hybrids; SHOEMAKER (**17**) arrived at the same conclusion with species of cultivated apples; LONGLEY (**9, 10**) discovered the stability of diploid species in *Crataegus* and *Rubus*, while "The polyploid species are characterized by striking irregularities in chromosome distribution and irregular pollen-formation, leading frequently to polycary and polyspory." Investigations on *Potentilla* (**14**) and many related genera indicated the same results. In *Gasteria*, TAYLOR (**21**) has indicated the hybrid origin of much of the material in cultivation, and questioned the authenticity of specific names as commonly used. There remains little doubt that the species studied is by no means pure, and perhaps some of the abnormalities can be explained on account of genetic weaknesses.

In connection with the regression of chromosomes, experiments

by CHAMBERS (2) are especially interesting. He demonstrated by microdissection that the reversion of jelly-like chromosomes to a more or less homogeneous sol could be brought about by mechanical injury. With fixed material, degeneration of quartet nuclei is described in several cases. In *Melilotus alba*, ROGERS (13) and CASTETTER (1) reported disintegration of three nuclei in quartets and the formation of giant pollen grains by the fourth nucleus. In *Carex* the same condition of disintegration of certain quartet nuclei and giant pollen formation is given by JUEL (5). From these observations it may be inferred that disintegration of nuclei is a normal phenomenon in certain species, and in some others it may be the result of mechanical injury.

KAUFMANN (6, 7), KUWADA (8), SHARP (16), and TAYLOR (26) have already offered lengthy reviews on the structure of large chromosomes. The discovery of spiral chromonemata and interpretation controverting the older investigators have also been recorded many times. It now seems that the structure of the larger chromosomes is understood to a degree. What remains to be investigated is the structure of smaller chromosomes. With the aid of new and more accurate technique, it is certain that many of the observations of larger chromosomes will be linked together into a coherent interpretation when the smaller chromosomes begin to attract the attention of the more skilled workers.

So far as behavior of the chromosomes is concerned, the peculiar cases of *Gasteria* recorded here are exceedingly interesting as well as perplexing. In their natural environment, such unusual behavior is perhaps being reported for the first time. However, judging from the abnormalities induced by chloral hydrate (15), excessive heat (18), and mechanical injuries (2), it is possible that some physiological factors are involved. On the other hand, it is probable that the material used is from a certain hybrid species of *Gasteria*, as shown by the polyspory and lagging of chromosomes at different stages of chromosomal development. It would be interesting from both physiological and cytological points of view, therefore, to conduct some parallel experiments with well controlled factors, whereupon it would not be difficult to learn some of the causes of the disturbance.

Summary

1. Behavior of normal reduction chromosomes of *Gasteria* as described by TAYLOR is cited and summarized.

2. Three types of abnormal meiotic and postmeiotic chromosomes of the same plant are described. One type, represented by closely coiled second metaphase chromosomes, is directly derived from the heterotypic chromosomes by an omission of the first interphase and second prophase. The other type, represented by giant headed chromosomes, is derived from the first metaphase chromosomes through a partial telophasic disorganization. The third type, represented by heavily constricted chromosomes in binucleated cells, is derived from the first metaphase chromosomes through a short lived interphase and breakdown of the nuclear membrane. The constrictions are much exaggerated and resemble the satellites in the somatic chromosomes.

3. The spiral which is involved in the structure of these types is single through the omission of the first interphase and second prophase.

4. Formation of third division chromosomes and 8-celled groups is described. This condition is thus far unique in higher plants.

5. Abnormal diakinetic chromosomes and chromosomes at other stages are able to assume a false resting stage after a brief process of disintegration of the chromatic material.

6. A nucleolus was seen in nuclei with fully developed chromosomes; it was not converted into any of the chromosomal appendages.

It is a pleasure to acknowledge the guidance of Professor WM. RANDOLPH TAYLOR, under whose supervision this study was conducted. The writer also wishes to express his hearty thanks to Professor CONWAY ZIRKLE and Dr. JOHN M. FOGG Jr. for their criticisms and suggestions regarding the manuscript.

TSING HUA UNIVERSITY
PEIPING, CHINA

[*Accepted for publication December 18, 1930*]

LITERATURE CITED

1. CASTETTER, E. F., Studies on the comparative cytology of the annual and biennial varieties of *Melilotus alba*. Amer. Jour. Bot. **12**:270–286. 1925.
2. CHAMBERS, R., Some physical properties of the cell nucleus. Science **40**: 824–827. 1914.
3. DARLINGTON, C. D., Chromosome studies in the Scilleae. Jour. Genetics **16**:237–251. 1925.
4. ERLANSON, EILEEN W., Cytological conditions and evidences for hybridity in North American wild roses. BOT. GAZ. **87**:443–509. 1929.
5. JUEL, H. O., Beiträge zur Kenntniss der Tetradenbildung. Jahrb. Wiss. Bot. **35**:626–659. 1900.
6. KAUFMANN, B. P., Chromosome structure and its relation to the chromosome cycle. I. Somatic mitoses in *Tradescantia pilosa*. Amer. Jour. Bot. **13**:59–80. 1926.
7. ———, Chromosome structure and its relation to the chromosome cycle. II. *Podophyllum peltatum*. Amer. Jour. Bot. **13**:355–363. 1926.
8. KUWADA, Y., On the spiral structure of chromosomes. Bot. Mag. Tokyo **41**:100–109. 1927.
9. LONGLEY, A. E., Cytological studies in the genus *Rubus*. Amer. Jour. Bot. **11**:249–282. 1924.
10. ———, Cytological studies in the genus *Crataegus*. Amer. Jour. Bot. **11**: 295–316. 1924.
11. MCCLUNG, C. E., Handbook of microscopical technique. Paul B. Hoeber Inc. New York. 1929.
12. NAWASCHIN, M., Morphologisches Kernstudium der Crepis-Arten in Bezug auf die Artbildung. Zeitschr. Zellforsch. Mikro. Anat. **2**:98–111. 1925.
13. ROGERS, W. E., Notes on *Melilotus alba*. Proc. Ia. Acad. Sci. **24**:415–482. 1917.
14. ROSCOE, MURIEL V., Meiotic irregularities in a *gigas* form of *Potentilla anserina*. BOT. GAZ. **84**:307–316. 1927.
15. SAKAMURA, T., Experimentelle Studien über die Zell- und Kernteilung mit besonderer Rücksicht auf Form Grosse und Zahl der Chromosomen. Jour. Coll. Sci. Imp. Univ. Tokyo **39**:1–221. 1920.
16. SHARP, L. W., Structure of large somatic chromosomes. BOT. GAZ. **88**:349–382. 1929.
17. SHOEMAKER, J. B., Pollen development in the apple, with special reference to chromosome behavior. BOT. GAZ. **81**:148–172. 1926.
18. STOW, ISAMU, Experimental studies on the formation of the embryo sac-like giant pollen grain in the anther of *Hyacinthus orientalis*. Cytologia **1**:417–439. 1930.
19. SVESCHNIKOVA, I. N., Karyological studies in *Vicia*. Bull. Appl. Bot. Genet. Pt. Breeding **17**:37–72. 1927.

20. TAYLOR, W. R., The smear method for plant cytology. BOT. GAZ. **78**:236–238. 1924.
21. ———, Cytological studies on *Gasteria*. I. Chromosome shape and individuality. Amer. Jour. Bot. **11**:51–61. 1924.
22. ———, The chromosome morphology of *Velthemia*, *Allium*, and *Cyrtanthus*. Amer. Jour. Bot. **12**:104–116. 1925.
23. ———, Cytological studies on *Gasteria*. II. A comparison of chromosomes of *Gasteria*, *Aloe*, and *Hawarthia*. Amer. Jour. Bot. **12**:219–223. 1925.
24. ———, Chromosome constrictions as distinguishing characteristics in plants. Amer. Jour. Bot. **12**:238–244. 1925.
25. ———, Chromosome morphology in *Frilillaria*, *Alstroemeria*, *Silphium*, and other genera. Amer. Jour. Bot. **13**:179–193. 1926.
26. ———, Chromosome studies on *Gasteria*. III. Chromosome structure during microsporogenesis and the postmeiotic mitosis. Amer. Jour. Bot. (In Press). 1931.
27. TUAN, H. C., A new method for safranin differentiation. Stain Tech. **5**:103–107. 1930.
28. ———, Picric acid as a destaining agent for iron alum haematoxylin. Stain Tech. **5**:135–138. 1930.

EXPLANATION OF PLATES I, II

All drawings were made with the aid of Zeiss 1.5 mm. apochromatic objective and ×20 compensation ocular, giving an approximate magnification of 4500.

PLATE I

FIG. 15.—Open spireme nucleus.

FIG. 16.—Nucleus at diakinesis stage.

FIG. 17.—Segment of diakinetic chromosomes showing first sign of regression.

FIG. 18.—Giant headed chromosomes showing structural detail.

FIG. 19.—Mid-telophase chromosomes showing loosely connected spirals.

FIG. 20.—First telophase chromosomes (*a*, *b*, *c*) showing origin of constrictions.

FIG. 21.—Early telophase nucleus showing loosening of spirals and formation of nuclear membrane at side near polar region.

FIG. 22.—Third division chromosomes (*a*, *b*, *c*, *d*) showing process of regression.

FIG. 23.—Late telophase spiral showing anastomoses and vacuolation.

FIG. 24.—Two sets of spiral chromosomes just before second metaphase.

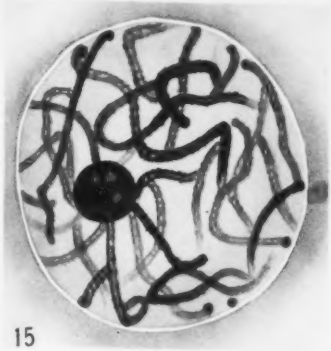
FIG. 25.—Second anaphase of spiral chromosomes.

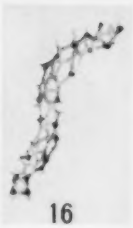
FIG. 26.—Second anaphase of giant headed chromosomes.

PLATE II

FIG. 27.—First metaphase chromosomes at equatorial plate stage.

FIG. 28.—Spirals in quartet nucleus, seven can be counted.


15


16

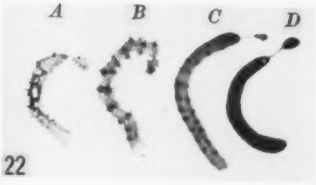
17

18

19

20

21

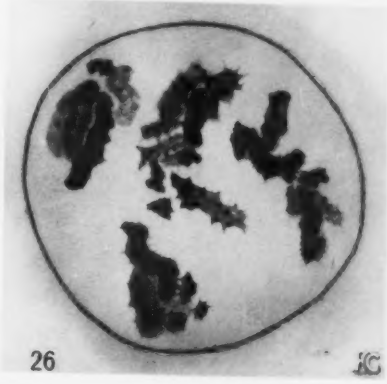


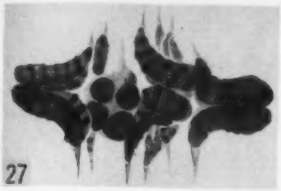
22

23

24

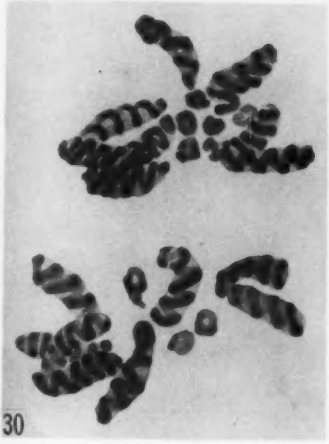
25


26


27

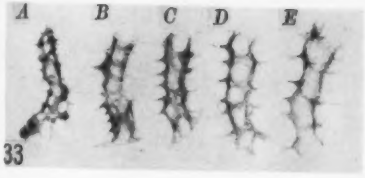
28

29


30

31

32

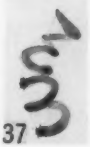


33

34

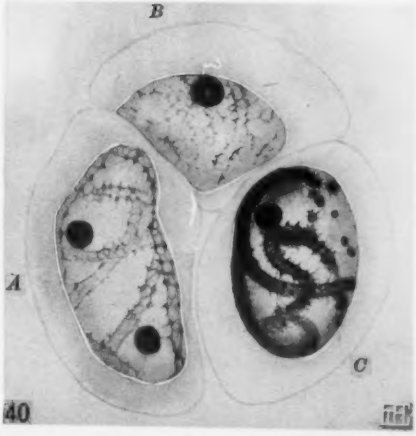
35

36


37

38

39



40

FIG. 29.—Chromatic thread from regression diakinetic chromosome.

FIG. 30.—Two sets of second metaphase dyads derived directly from first metaphase tetrads showing spiral structure in each of chromatids.

FIG. 31.—Late anaphase daughter group showing separation of dyad partners.

FIG. 32.—Tetrad threads from regression diakinetic chromosomes.

FIG. 33.—Selected second telophase giant headed chromosomes (*a*, *b*, *c*, *d*, *e*) showing disorganization of chromatin material; head still recognizable in *a*.

FIG. 34.—Spirals from quartet nucleus.

FIG. 35.—Seven continuous spirals from another quartet nucleus.

FIG. 36.—Quartet nucleus showing regression of third division chromosomes.

FIG. 37.—First telophase spiral showing anastomoses.

FIG. 38.—Same as fig. 37.

FIG. 39.—Quartet nucleus at early telophase, seven spirals can be counted.

FIG. 40.—Same as fig. 36; *b* shown at resting stage.

DISCHARGE OF SPERMS IN MARCHANTIA DOMINGENSIS

EMMA N. ANDERSEN

(WITH NINETEEN FIGURES)

Introduction

The different statements occurring in textbooks and in research papers, concerning the discharge of antheridia, made it seem desirable to study the antheridia of *Marchantia*, to find if possible the mechanism which discharges the antherozoids.

THURET (**17**) in 1856 observed the forcible discharge of sperms in *Conocephalus conicus*. PEIRCE (**15**), working later, stated that the mechanism effecting expulsion of the sperms in *Asterella californica* consists of two parts: (1) the water-absorbing matrix consisting of gelatinized mother cells with thin walls, in which the sperms lie, which distend the mature antheridium still inclosed by the single layer of peripheral cells; (2) the thin walled, large celled, water-absorbing tissue which forms the walls of the chambers in which antheridia develop. These two, the tissue and the water-absorbing matrix, expand in opposite directions as they absorb water, the former tending to decrease and the latter to increase the size of the chambers containing antheridia. The two pressures would tend to offset each other and affect nothing if they met on all sides of the antheridia. The chambers in which the antheridia lie are open above, and are covered by the chlorophyll-containing, smaller celled, and more rigid tissue. The antheridia are distended in all directions and are compressed on all sides except from above. PEIRCE concluded that the distending and compressing strains finally result, under these considerations, in the rupture of the antheridium and the explosive discharge of its contents through the mouth of the chamber.

CAVERS (**3**), working on the same genera, observed that explosive discharges took place on warm, sunny days, and that they were especially frequent when the plants were exposed to direct sunlight; they were not observed on dull days, nor when the plants were shaded. He accounts for the pressure resulting in the expulsion of

the antherozoids as caused by water absorbed by various mucilage cells; and if any antheridia are ripe, the walls of the antheridium as well as of the sperm mother cells, which at this time are largely mucilaginous, also take up water and become swollen. KING (9), who also worked on *Conocephalus*, stated that moisture alone may be the inciting cause of the explosion; his statement was based on observations made in the afternoon after spraying.

CAMPBELL (1), in his treatment of antheridia, says that the mature walls of the sperm cells become mucilaginous; and when the mature antheridium is wet, the swelling of this mucilaginous mass bursts open the antheridium and sets free the sperm cells, from which the spermatozoids are liberated by complete dissolution of the cell wall. STRASBURGER (16) states that the spermatozoid mother cells are ejected from the antheridium.

GOEBEL (7) observed in the Marchantiaceae a great increase of radial diameter of the cells of the wall of the deeply sunken mature antheridia. Here the expansive pressure was against the wall of the cavity in which antheridia are located.

The opening of the cavity in many forms is drawn into a point projecting over the upper layer. GOEBEL thinks this serves the same purpose as the point of a sprayer; the antheridial wall acts as the wall of the rubber bulb when squeezed. In many liverworts the ejection of spermatozoids occurs as if from this apparatus.

Discharge of the antheridia of *Marchantia domingensis* in free-hand sections of the antheridial branches was observed under the low power of the microscope. The great inward distention of the antheridial wall cells, with the exception of the cells at the apex and the highly colored cells of the disk lining the antheridial chamber, was very striking. The cause of this distention was sought; and a brief study was made of the development of the cells of the antheridial wall, the chemical nature of the cell walls and cell contents of the antheridia, and the antheridial branches, in an effort to learn more about the mechanism of the discharge of the antherozoids.

Materials and methods

Plants of *Marchantia domingensis* were grown for a number of years in flats in the greenhouse at a temperature of approximately

22° C., and watered daily. The thalli grow luxuriantly, and at first produce only cupules (fig. 1). A very few antheridia can also be seen. Later abundant antheridial branches are produced, and when these reach the size shown in fig. 2, the antheridia near the center of the disk are discharging the antherozoids. The work was done on antheridial branches of this age. Later, when the antheridia have discharged the antherozoids, the antheridial disks are much greater in diameter and are more deeply lobed. In fig. 3 they are shown with archegonial branches. The old antheridia lie close to the thalli, and their disks have a greater diameter than the archegonial disks.

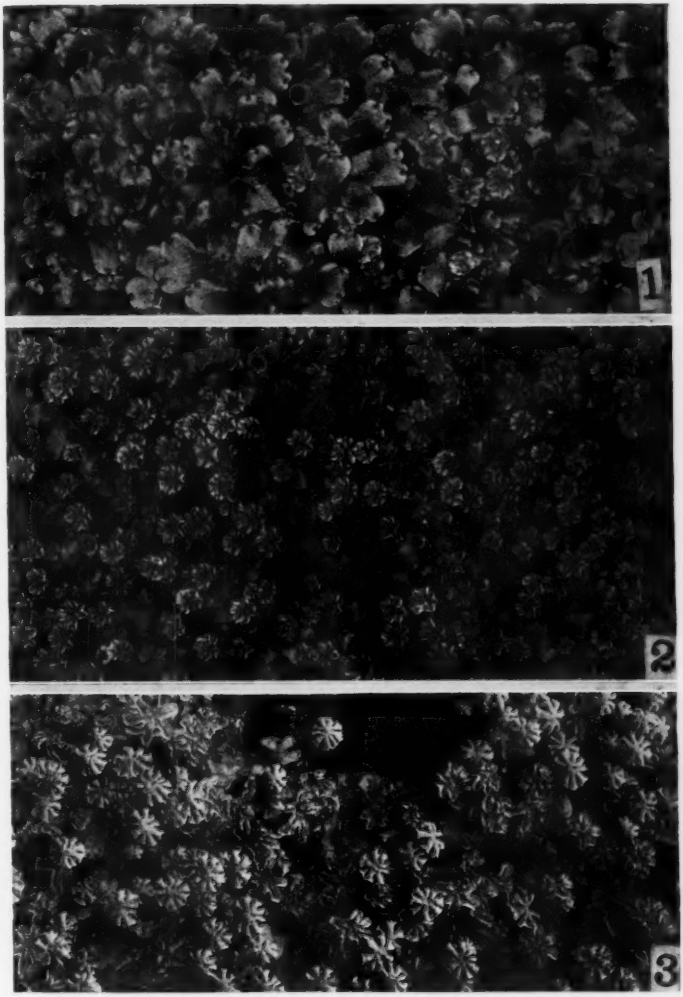
Freehand sections of living material, made with a razor and freezing microtome sections, were used in the microchemical study. A brief outline of each microchemical test is given where the substance investigated is discussed in this paper. Details of tests when not given may be found in ECKERSON (4), TUNMANN (18), MOLISCH (12), and MANGHAM (11). The tests as outlined by ECKERSON are used for the most part.

Material was fixed in the following solutions: acetic alcohol, Carnoy's, chromo-acetic, Flemming's, formalin alcohol, formalin acetic alcohol, and platinum chloride. The material was dehydrated in the usual manner up to 70 per cent alcohol. Free-hand, freezing microtome and paraffin sections were made from material in 70 per cent alcohol in the study of the development of the cells of the antheridial wall. Material fixed in Flemming's, Carnoy's, platinum chloride, and formalin acetic alcohol when sectioned proved to be satisfactory. Photomicrographs[1] were taken of free-hand sections fixed in Flemming's stronger solution, sectioned from 70 per cent alcohol and mounted in glycerin on the slide (figs. 4–9).

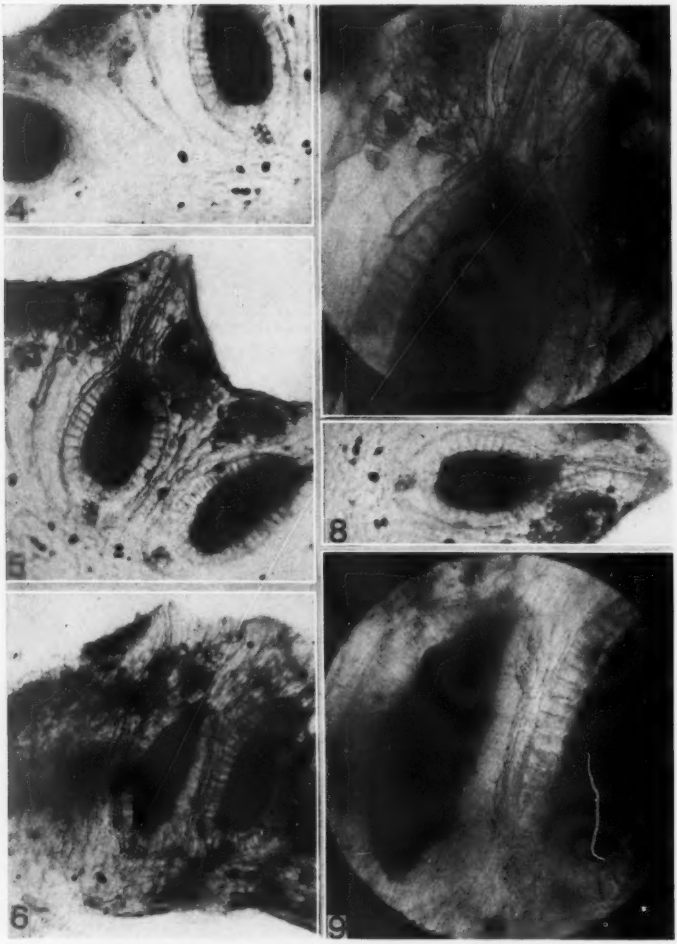
Investigation

The antheridial branches of *M. domingensis*, as described for the genus *Marchantia* (2, 5, 16), consist of a lobed, horizontal disk and erect stalk. The disklike thallus is dorsiventral, and consists of three regions: upper epidermal, chlorophyll bearing, and lower compact part (fig. 5). The chlorophyll region and the turgid cells

[1] The writer wishes to express her appreciation to Dr. ELDA R. WALKER for aid in photomicrographic work, to Dr. SOPHIA H. ECKERSON for suggestions, and to Professor T. J. FITZPATRICK for reading manuscript and proof.



FIGS. 1–3.—Fig. 1, thallus showing cupules and very few antheridia; fig. 2, antheridial branches and cupules; fig. 3, old antheridial branches and archegonial branches; ×1.



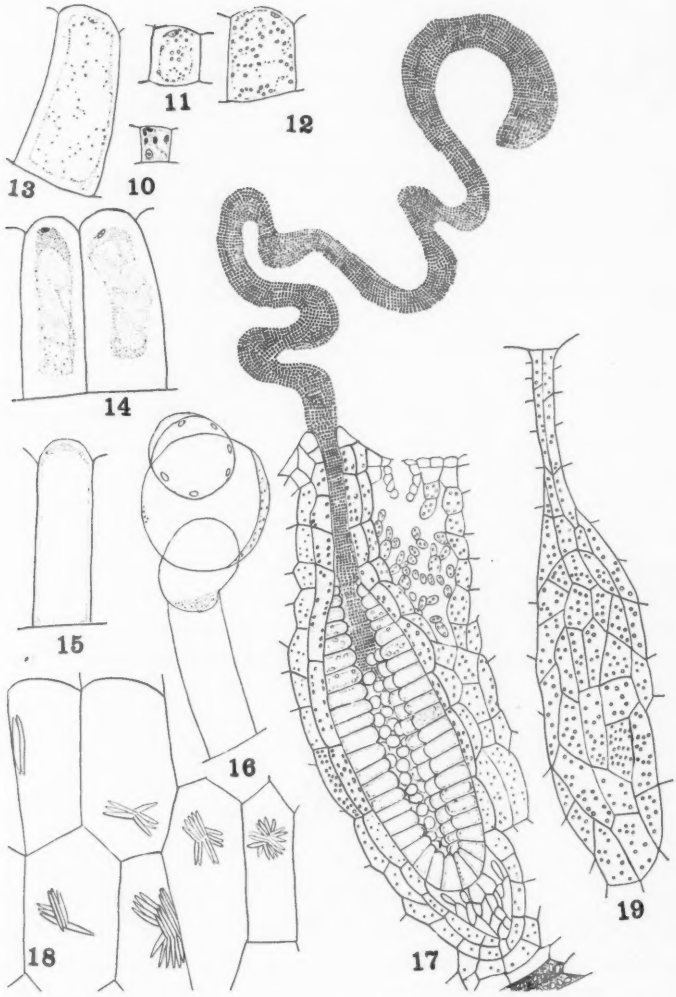
FIGS. 4–9.—Photomicrographs of mostly immature antheridia (dark area in center is spermatogenous mass): Fig. 4, immature antheridium at left, antheridium about to discharge at right, ×45. Figs. 5, 7, 8, photosynthetic chambers and turgid cells lining antheridial cavity, figs. 5, 8, ×45; fig. 7, ×100. Fig. 6, different stages of development; antheridium at extreme left has discharged contents and collapsed, ×40. Fig. 9, one to left showing transparent condition of wall cells at discharge; ×85.

lining the antheridial cavity are shown in fig. 7. Scales and rhizoids occur on the ventral surface of the disk. The antheridia arise singly in flasklike depressions which extend down into the compact tissue. The cavities or depressions are surrounded by air chambers containing branched rows of photosynthetic cells and epidermal pores. Antheridia do not all mature at once, the oldest being near the center. The antheridia are stalked (figs. 4, 5, 8), and contain a mass of spermatogenous cells inclosed by a wall consisting of a single layer of sterile cells. In figs. 4–9 the spermatogenous mass appears black, and all antheridia show walls whose cells have begun to elongate inwardly, except the antheridium on the left in fig. 4. In the cavities surrounding the stalk are turgid cells or paraphyses.

In free-hand sections of antheridial branches of *Marchantia domingensis* and *M. polymorpha*, it is possible to obtain them sufficiently thin to enable one to see antheridia in their chambers. It was observed under low power, that many of the mature antheridia soon after being placed in water on the slide began to discharge their contents, which consisted of sperm mother cells containing sperms or antherozoids instead of free sperms. The biciliated sperms or antherozoids rotated rapidly in the mother cells as they were ejected, but the sperms were not free to swim and escape from the mother cell while in the antheridium. Although many antheridia were injured in mounting and sectioning, there were always a sufficient number of uninjured ones apparently behaving normally. When conditions were optimum the sperm mother cells were ejected in a relatively short time in smokelike columns.

In young antheridia of *M. domingensis*, the cells of the wall are more or less cubical, and contain a nucleus and a number of chloroplasts lying in the cytoplasm (fig. 10).

When the antheridia have reached an intermediate stage in growth and an almost full-grown but not mature stage, the cells of the antheridial wall have become elongated, and apparently many chloroplasts have begun to disintegrate (figs. 11, 12). After disintegration granules are present (fig. 13), which become blue when iodin is added, indicating the presence of much starch. At the time of dispersal, the cells of the antheridial wall contain most frequently only granules imbedded in the cytoplasm (fig. 13). Occasionally at



FIGS. 10–19.—Figs. 10–16, development of cells of antheridial wall; ×450. Fig. 17, antheridium discharging smokelike column of sperm mother cells; ×120. Fig. 18, probably maltosazone crystals in tissue below antheridia; ×450. Fig. 19, pigmented cell lining antheridial chamber; ×120.

dispersal some chloroplasts are still intact, as shown in the upper part of the antheridium in fig. 17 and the uppermost droplet in fig. 16. Paraffin sections of antheridia whose walls are distended inwardly often show a nucleus (fig. 14). Distended cells of the wall which contain no nuclei occasionally are found in paraffin sections (fig. 15), but nuclei were never seen in living discharging antheridia (figs. 16, 17).

At the stage when the antheridia are fully mature and eject their sperm mother cells when placed in water, the cells of the wall are greatly distended, with the exception of those at the apex (figs. 5–9). The cells of the wall distend to such an extent that they ultimately almost fill the spermatogenous cavity. They contain fewer granules, indicating that little starch is present. The antheridia are kept from receding in the chamber by a number of turgid paraphyses, surrounding the antheridial stalk at the base of the chamber (fig. 17).

The sperm mother cells have also increased in size. They appear to be pushed out slowly in smokelike columns by the greatly distended wall cells which have elongated inwardly, making the spermatogenous cavity smaller. The cells lining the entire antheridial chamber, or usually more than the upper half, are now highly colored, red predominating (fig. 19). They turn a bright red when treated with acid, and when treated with dilute alkali they become blue, indicating the presence of anthocyanin. Freezing microtome sections of the cells lining the antheridial chamber show that the walls are colored uniformly, the corners usually appearing more deeply colored. The cells contain many chloroplasts. Attempts were made to determine whether or not the cell sap of these cells also contained anthocyanin, but the results were always negative. NAGAI (13), however, states that not only the walls of *M. polymorpha* but also the cell sap contained anthocyanin. The change in color of the cells lining the antheridial chamber no doubt bears a significant relation to the chemical nature of the cells of the antheridial wall, as well as to their contents and to the ultimate discharge of the spermatogenous mass. ONSLOW (14) states that one idea is that red pigment assists the diastase activity by screening it from deleterious rays, thus facilitating the hydrolysis of starch and subsequent translocation. Those antheridia of *M. domingensis* representing an inter-

mediate stage of growth contain relatively large amounts of starch in the cells of the antheridial wall, while the mature antheridia, screened by the reddish cells of the wall of the chamber, contain relatively small amounts. It may be that the decreased starch content in mature antheridia is due to the influence of the anthocyanin cells lining the chamber; on the other hand, the pigmented cells may prove to be merely a mechanical device to aid inward distention of cell walls of the antheridial wall and the subsequent discharge of sperm mother cells. That there is a greater need for a stronger upper lining of the antheridial chamber than the lower, is because the upper part is adjacent to the air chambers containing branched rows of photosynthetic cells, thus giving little support to the antheridial lining (figs. 5, 7, 17).

As soon as most of the spermatogenous material has been expelled, hyaline drops become apparent. The writer first thought that these drops were plastids greatly enlarged, since chloroplasts in ruptured cells of the disk have been observed to be much swollen when water enters the cells. ZIRKLE (**20**) believes that the swelling of chloroplasts of fern prothallia when water enters a cell is due to imbibition of water by the contents of the central vacuole of the chloroplast, which remains separated from the outer water by a surface film. Upon further observations, the writer noted that the apices of the cells of the antheridial wall of *M. domingensis* near the center of the antheridium appear to dissolve. As these walls dissolve the cell contents escape in one or more spheres or films. In the process of exit from the cells (fig. 16) these drops are more or less compressed, but upon being freed they assume a spherical form. These droplets are ephemeral and sooner or later burst. Emerging droplets do not constitute a rare phenomenon, since many workers have observed films about droplets of protoplasm which have emerged from cells (**8**). The droplets probably consist of cytoplasm, broken down chloroplasts, and dissolved wall material. Each droplet appears to contain a large vacuole. It was often observed that the greater portion of the granular material is attached to one side of the droplet, like the granular stroma of swollen chloroplasts. Very rarely was a drop seen in which the plastids were still intact. This condition is shown in the uppermost drop (fig. 16). The droplets are exuded into

the spermatogenous cavity and at this time are much distended. They appear to aid in expelling the few remaining sperm mother cells. GARDINER and ITO (**6**), investigating the structure of the mucilage secreting cells of *Blechnum occidentale* and *Osmunda regalis*, report mucilaginous hyaline drops and droplets. Each drop had its own protoplasmic reticulum and contained tannin. The spermatogenous cells of *M. domingensis* indicate a slight trace of tannin, but none was detected in the cells of the antheridial wall.

Microchemical tests and results

MEMBRANES

MUCILAGE.—According to KRAEMER (**10**), cellulose walls may be divided into the following groups: ligno-cellulose walls, protective cellulose walls, reserve cellulose walls, mucilage cellulose, and mineral cellulose walls. He speaks of mucilage cellulose walls consisting of cellulose and mucilage as found in all parts of the plant, and states that they dissolve or swell in water and are colored blue or yellowish with iodin. They are stained also with alcoholic or glycerin solutions of methylene blue. The antheridia of *Marchantia domingensis* were treated with iodin, but the cell walls of the distended antheridial wall cells were colored neither blue nor yellowish unless treated for several hours. The spermatogenous mass became very yellow. Each mother cell wall, however, had only a slight trace of color if any.

YOUNGKEN (**19**) also states that mucilage may be demonstrated in plant tissues by placing sections in a deep blue solution of methylene blue in equal parts of alcohol, glycerin, and water on a glass slide, allowing them to remain in the solution several minutes, and then draining and mounting them in glycerin. The cells containing mucilage should exhibit bluish contents. The results from this method indicated the presence of mucilage in the cell walls of the antheridia. The cell walls of the antheridial branch are colored blue. Chlorzinc iodide colors the cell walls of the antheridial branch and young antheridial walls. Usually, however, a thick inner layer which stains less deeply is formed (fig. 11) in the antheridial wall cells. Later, the part of the layer that extends from the center to the apex deepens in color (fig. 13). It is probably mucilage.

The following tests for mucilage, used by GARDINER and ITO (**6**),

were also used. They found that alcohol caused abnormal shrinking of the mucilage and was attended by great distortion of structure. While alcohol always seemed to arrest activity of the cells of the antheridial wall of *M. domingensis*, they were never greatly distorted. Saturated watery picric acid produced great swelling of the mucilaginous contents, which finally ended in bursting the cell and permitting the mucilage to escape. While sections of the antheridial branches were used, attention was again focused on the cell walls of the antheridial wall with negative results. With iodin, mucilage stained a clear yellow. The antheridial branches of *M. domingensis* were sectioned and used, and only the spermatogenous mass appeared yellow. A 2 per cent chromic acid solution was used on some sections and a 1 per cent osmic acid solution on other sections of antheridial branches. Since but a trace of tannin had been obtained in the sections, these tests were also negative.

HEMICELLULOSE.—Sections of antheridial branches were heated in 3 per cent sulphuric acid over a water bath for 2 hours. The walls neither completely nor partially hydrolyzed, indicating that hemicellulose was not present. Sections of the antheridial branch were treated with warm water, alcohol, ether, and then heated with an excess of concentrated nitric acid and placed in a moist chamber. Mucic acid crystals, indicating the presence of galactan, could not be detected.

Sections of antheridial branches were placed on a slide in a 4 per cent orcin solution, after which a drop of concentrated hydrochloric acid was added. They were then heated to the boiling point. After boiling, the antheridia were usually a structureless mass. The blue or violet color apparently occurs in the cell walls of the spermatogenous mass, and sometimes it seems to appear in cells of the antheridial wall, in cell walls at the edge of the disk and stalk, and in the portion of the disk under the antheridial chambers. A blue or purple color indicates the presence of pentoses. Sections of the male branch were placed on slides and treated with a 1 per cent solution of phloroglucin, after which a drop of concentrated hydrochloric acid was added. They were then heated for ten minutes. As in the former case, the antheridia became structureless. The same walls were colored red, again indicating the presence of pentoses.

CELLULOSE.—Sections of antheridial branches were treated with

75 per cent sulphuric acid and iodin potassium iodide. The cell walls of the cells of the epidermal and chlorenchymatous tissue in the upper part of the disks, of the regions between and below antheridia, of the scales and rhizoids, and of the stalk of the antheridial branch (with the exception sometimes of a few small interrupted areas along the edge of the disk) were colored blue, which indicates the presence of cellulose. The cell walls of the immature antheridial wall were blue, but only a trace was found in sperm mother cell walls. Sections were also observed with polarized light, and the same walls were doubly refractive. Chlorzinc iodide was also used. It confirmed the previous test and in addition colored the epidermal cells of the antheridial branches and outer tangential cell walls of the antheridial wall yellow, indicating the presence of cutin.

PECTIC SUBSTANCES.—Sections of the male branch were placed on a slide in a dilute solution of ruthenium red. Excluding the antheridia, the cell walls of the remaining cells of the disk and stalk of the antheridial branch stained red, which indicates the presence of pectic substances. Sections of the male branch were heated in 2–15 per cent hydrochloric acid over a water bath for 0.5–2.5 hours. At the end of that time, after a thorough washing, the cell walls of the antheridial branch excluding the antheridia stained as well as at the initial staining, with the exception of the rhizoids and some scales. Some of the scales dissolved completely, indicating that they consisted entirely of pectose.

Sections of the antheridial branch were heated in 2 per cent ammonia for one hour. They stained as well as at the initial staining, indicating again that the walls are not pectic acid but pectose or calcium pectate, and must first be treated with a weak solution of hydrochloric acid. Sections of the antheridial branch were heated in 2–15 per cent hydrochloric acid from 15 minutes to one hour, thoroughly washed, and then heated in 5 per cent ammonium oxalate for 30 minutes to one hour. The cell walls of the antheridial branch (excepting antheridia) no longer stained, indicating that the walls contain calcium pectate.

STORAGE SUBSTANCES

STARCH.—Iodin potassium iodide solution was used. The cells of the chlorenchymatous portion of the disk and of the regions between

and under the antheridia, the anthocyanin cells lining the cavity of the antheridia, and the cells of the upper part of the stalk of the antheridial branch usually contain abundant starch. Disks occur, however, in which little or no starch is found except in antheridia, or only in the chlorenchymatous portion with a little in antheridia. Other disks occur where starch is found largely in the tissue between and under the antheridia, together with a little in a few of the antheridia. Again other disks may be found in which starch occurs only in the layer immediately under the antheridia, with some in a few of the antheridia. Starch when it occurs in antheridia is always found in the cells of the antheridial wall. The youngest antheridia near the periphery of the disk rarely if ever contain starch, and the discharging ones usually also contain very little. The cells of the walls of the remaining antheridia, representing the intermediate stages, contain starch in varying amounts. Of these, the antheridia nearest the maturing ones contain the least.

SUGARS.—Antheridia of all ages were treated with 15–20 per cent sodium hydroxide and copper tartrate, and then heated (Flückiger's test). No reduction was obtained on gently warming or without heating, indicating that probably fructose was not present. Upon longer heating crystals of cuprous oxide were observed, however, which may indicate that glucose was present. Crystals were also observed in the cells above and below the antheridia, when branches were tested.

The phenylhydrazine hydrochloride and sodium acetate test was also used. On some slides antheridia alone were placed in the solution, and on others sections of the antheridial disk as well as antheridia were used. Some sections were heated, others were kept at room temperature for two days, and neither fructosazone nor well formed glucosazone crystals were obtained in the cells with or without heating.

Sections which gave no crystals after two days at room temperature were heated on a water bath for about 40 minutes, to see whether crystals would form. None formed, thus indicating the absence of sucrose.

Sections were heated 15, 35, 45, 60, and 75 minutes respectively, and examined every few days over a period of four months (11).

Sections which had been heated 45 minutes on a boiling water bath gave good crystals in about one month. More crystals had formed in two months. At the end of three months many sections that had been heated one hour in the reagent now also contained good maltosazone crystals (fig. 18). At the end of four months no increase in any region could be ascertained. Many of the immature antheridia which had not yet elongated their cell walls were covered with crystals, probably indicating diffusion of sugar through the walls; but the mature antheridia which had discharged their contents contained very rarely more than one cluster of crystals. In many of the disks, the cells constituting the layer beneath the antheridia, as well as the cells between the antheridial chambers, contained maltosazone crystals. Similar large clusters of crystals commonly occur on the exterior portion of the disk. As in the case of the young antheridia, I have interpreted the crystals as having been formed from sugar diffused from the chlorenchymatous tissue. Since the cell walls of the wall cells and mother cells of the antheridia just before discharge consist largely of a substance which gives the pentosan reaction, and the cells of the antheridial wall contain little glucose if any, and also a small amount of maltose, it would seem that if osmosis indeed enters into the mechanism of spermatogenous discharge, it plays but a minor rôle.

Mineral substances

Sections of antheridial disk were treated with 1 per cent benzidine in 3 per cent hydrochloric acid, and many needle crystals of benzidine sulphate were obtained. The storage region of the disk located below the antheridial chambers contained abundant crystals of sulphate. Antheridia and chlorenchymatous tissue as well as scales and rhizoids gave negative results.

In treating sections of antheridial disk with a saturated solution of ammonium chloride in water, with a few drops of ammonia and sodium phosphate crystals to make a 0.1 per cent solution, a few ammonium-magnesium-phosphate crystals appeared in the antheridia. The cells of the antheridial wall coalesce completely upon warming, but in spite of this fact the magnesium crystals are primarily found only in the peripheral cells of the antheridium. The significance of magnesium in the cells of the antheridial wall is not clear.

It may have been derived from the broken-down chloroplasts, and its presence may change the viscosity of the protoplasm, slightly shortening the existence of extruded drops. Other mineral tests gave negative results.

Discussion

In viewing the discharge of *Marchantia domingensis*, the great inward distention of the cells composing the antheridial wall, with the exception of the apical cells is the striking feature. Since this distention might be due to osmotic pressure, sugar tests of the cells of the walls were made. All the tests used were microchemical, in order to obtain localization. While there were immature antheridia which had not yet elongated their walls, and which were covered with crystals, the antheridia which had discharged their contents contained, if any, rarely more than one cluster of maltosazone crystals. The pentose reaction was obtained in the cells of the sperm mother cells and sometimes in the antheridial wall, but the reaction for mucilage occurred in both. The behavior of the antheridial wall, however, was that of a pentosan in having the power of swelling and of taking up a large quantity of water. It is also mucilaginous in character like the pentosans. The cell walls of the young antheridial wall also gave the cellulose reaction, and the exterior or surface walls were covered besides with a thin layer of cutin. The theory that osmotic pressure was the cause of the great inward distention was abandoned when the cells of the antheridial wall indicated that they were principally mucilage or pentosan, and contained little sugar.

While discharging antheridia contained little starch, those in intermediate stages often contained a great amount; of the immature antheridia, those nearest maturity usually contained the least. Since the cells lining the antheridial chambers become colored at approaching maturity, red predominating, it was thought that those cells might influence chemical activities occurring in the cells of the antheridial wall. If they increase the temperature of the cells of the antheridial wall, there might be increased pentosan formation. This red pigment or anthocyanin, found in the cells lining the antheridial cavities, might also facilitate the hydrolysis of starch, and thus account for the decreased amount of starch usually found in antheridia that are discharging their contents. While it seems probable

that the pigmented cell walls of the antheridial chamber may affect these chemical activities, on the other hand it may prove to be a mere mechanical device for aiding sperm mother cell discharge. The

TABLE I

SUMMARY OF MICROCHEMISTRY OF CELLS OF ANTHERIDIAL BRANCH OF MARCHANTIA DOMINGENSIS

MEMBRANES	
MUCILAGE	**PENTOSANS**
Cell walls of antheridial wall and sperm mother cells	Cell walls of spermatogenous cells and sometimes antheridial wall cells Cell walls at edge of branch Cell walls of ventral region of disk
CELLULOSE	**PECTIC SUBSTANCES**
Cell walls of immature antheridial wall cells All cell walls of antheridial branch with trace in spermatogenous wall cells and with exception sometimes of a few small interrupted areas along edge of disk	All cell walls of stalk and disk of antheridial branch with exception of antheridia
	PECTOSE
	Cell walls of scales and rhizoids
CUTIN	**CALCIUM PECTATE**
Epidermal cells of antheridial branch Outer tangential cell walls of antheridial wall	All cell walls of stalk and disk of antheridial branch with exception of antheridia, scales, and rhizoids
STORAGE SUBSTANCES	
STARCH	**SUGARS**
In plastids of cells of photosynthetic cells in air chambers In plastids of anthocyanin cells and cells between and under antheridia In plastids of cells in upper part of stalk In plastids of antheridial wall cells	**GLUCOSE** Cells of antheridial wall Cells between and below antheridia **MALTOSE** Cells of antheridial walls Cells between and below antheridia Cells of epidermal area
MINERAL SUBSTANCES	
SULPHATE	**MAGNESIUM**
Cells of the ventral region of disk	Cells of the antheridial wall

most important single factor occurring in the spermatogenous discharge seems to be the enormous inward distention of the cells of the antheridial wall, although several other factors aid in the discharge.

The turgidity of the cells lining the cavity causes the cells of the

antheridial wall to distend toward the interior of the cavity, bringing considerable pressure to bear on the spermatogenous mass. At the same time the sperm mother cell walls are also increasing in size, resulting in their expulsion in smokelike columns. Basal cells or paraphyses surround the stalk of the antheridia, and prevent them from receding into the cavity. They aid in maintaining the contact between the apex of the antheridium and the opening that leads to the surface of the disk, thus facilitating discharge.

Finally, in the cells of the antheridial walls a katabolic process is taking place, which results in the formation of hyaline drops, probably composed of cytoplasm, broken-down plastids, and some of the cell wall substance. These hyaline drops, usually ephemeral, are discharged into the spermatogenous cavity; and as they fill the cavity, which by this time is much diminished in size, they seem to aid in expelling the few remaining sperm mother cells. The mass of spermatogenous material ejected consists of sperm mother cells which contain the sperms. The sperms can be seen rotating rapidly for some time in each cell. Eventually complete dissolution of the cell takes place and the sperms are free to swim.

Table I summarizes the microchemistry of the cells of the antheridial branch.

Summary

1. In *Marchantia domingensis*, the cell walls of the cells of the antheridial wall and of the sperm mother cells, before discharge consist largely of mucilaginous material which gives the pentosan reaction in the sperm mother cells, and sometimes in the antheridial wall cells. The cell walls of the latter when immature also give a cellulose reaction, and the exterior or surface walls are covered with a thin layer of cutin.

2. Excluding the antheridia, almost all of the cell walls of the disk and stalk of the antheridial branch, as well as the scales and rhizoids, give both a cellulose and a pectic reaction.

3. Sugar reactions in the antheridial peripheral cells or wall cells by means of Flückiger's test are questionable. Glucose may be present.

4. The greatest number of maltosazone crystals is found on the exterior region of non-mature antheridia. Maltosazone crystals are

obtained in many cells of the disk between the antheridia, and also in cells occupying the region below them.

5. The cell walls of the cells lining the entire or a little more than the upper half of the antheridial cavity contain anthocyanin at maturity, which may tend to increase diastase activity, katabolic activity, and incidentally also pentosan formation; on the other hand the pigmented lining may function wholly in a mechanical capacity.

6. The significance of magnesium in the cells of the antheridial wall, as well as the abundance of sulphate in the ventral region or in the layer of cells beneath the antheridia, is not clear. The magnesium may change the viscosity of protoplasm.

7. Discharge of the spermatogenous mass depends upon: (1) the inward distention of the cells of the antheridial wall caused by the large amount of water imbibed; (2) the simultaneous swelling of the spermatogenous cell walls; (3) the pressure exerted by the turgid more or less pigmented cells lining the antheridial cavity and causing its gradual diminution as the spermatogenous mass is being ejected in smokelike columns; (4) the tendency of the swollen basal cells or paraphyses in the antheridial chamber to keep the antheridium in its upright position; (5) the force emitted by expelled hyaline drops probably composed of cytoplasm, broken down plastids, and wall substance.

8. In the ejection of spermatogenous material the cells of the antheridial wall play a major part.

UNIVERSITY OF NEBRASKA
LINCOLN, NEB.

[*Accepted for publication July 25, 1930; delayed through author's revision*]

LITERATURE CITED

1. CAMPBELL, D. H., A University textbook of Botany. 1902.
2. ———, The structure and development of mosses and ferns (Archegoniatae). 3d ed. New York. 1918.
3. CAVERS, F. I., Explosive discharge of antherozoids in *Fegatella conica*. Ann. Botany **17**:270–274. 1903.
4. ECKERSON, SOPHIA H., Mimeographed outlines of methods of microchemistry.

5. EVANS, H. W., Transactions of the Conn. Acad. of A. & S. **21**:201–313. 1917.
6. GARDINER, W., and ITO, TOKUTARO, On the structure of the mucilage secreting cells of *Blechnum occidentale* L. and *Osmunda regalis* L. Ann. Botany **1**:27–54. 1887.
7. GOEBEL, K., Organographie der Pflanzen. III Aufl. II Teil. Jena. 1930.
8. HEILBRUNN, L. V., The colloid chemistry of protoplasm. Berlin. 1928.
9. KING, C. A., Explosive discharge of antherozoids in *Conocephalum*. Torreya **3**:60–61. 1903.
10. KRAEMER, H., Textbook of botany and pharmacognosy. 4th ed. 1916.
11. MANGHAM, S., Observations on the osazone method of locating sugars in plant tissues. Ann. Botany **29**:369–391. 1915.
12. MOLISCH, H., Mikrochemie der Pflanze. Jena. 1913.
13. NAGAI, I., Über rote Pigmentbildung bei einigen Marchantiaarten. Bot. Mag. Tokyo **29**:90–98. 1915.
14. ONSLOW, M. W., The anthocyanin pigments of plants. 2d ed. Cambridge. 1925.
15. PEIRCE, G. J. I., Forcible discharge of antherozoids in *Asterella californica*. Bull. Torr. Bot. Club **29**:374–382. 1902.
16. STRASBURGER, ET AL., Textbook of botany. 5th ed. New York. 1921.
17. THURET, G., Discharge of antherozoids in *Fegatella*. Mem. Soc. Sci. Nat. Cherbourg **4**:216. 1856.
18. TUNMANN, O., Pflanzenmikrochemie. Berlin. 1913.
19. YOUNGKEN, H. W., Pharmaceutical botany. 4th ed. Philadelphia. 1923.
20. ZIRKLE, C., The structure of the chloroplast in certain higher plants. Amer. Jour. Bot. **13**:321–341. 1926.

MARCESCENT LEAVES OF CERTAIN SPECIES OF QUERCUS

EARL E. BERKLEY

(WITH ELEVEN FIGURES)

The abscission of leaves was first studied by VON MOHL (**5**); since then considerable work has been done, although little attention has been given to the phenomenon of marcescent leaves. TISON (**4**) observed both marcescent and deciduous leaves in *Carpinus betulus*, *Fagus sylvatica*, *Quercus hispanica*, and *Q. pedunculata*. In the deciduous leaves the abscission layer was formed in the autumn, followed by the ligno-suberization of the other cells of the scar, giving rise to a protective layer. In the petioles of the marcescent leaves, only partially formed abscission layers were found, marked by the breaking apart of small groups of cells as a result of gelatinization of their walls. For the most part the marcescent leaves were situated on the short lateral branches, and it was thought that the marcescent condition was induced by injury due to frost or freezing, which suddenly killed the petiole and thus prevented further development of the abscission layer. The marcescent leaves were abscissed the following spring by the development of an abscission layer just beneath the ligno-suberized area. A similar meristematic region was formed just beneath the protective layer of the stubs left after the leaves had fallen. LEE (**1**) confirmed TISON's work on *Carpinus*, but he did not investigate the marcescent leaves of *Quercus*.

LLOYD (**2, 3**) observed marcescent leaves on a number of forest trees. He did not examine petioles of all species observed, but found partially formed abscission layers in some of the species examined. In further work on cotton, he observed that in certain varieties the bolls died and withered on the stem without falling.

In the late autumn of 1927, the fact that the leaves of a certain oak tree did not fall attracted the attention of the writer. Observations were continued throughout the ensuing winter, and during the period extending from May, 1928, to May, 1929. Collections of

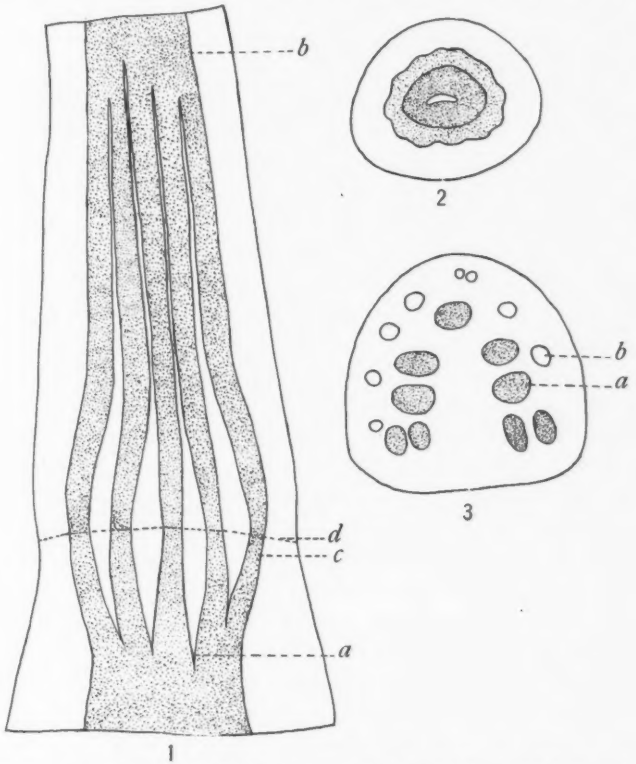
petioles from various species of *Quercus* were taken at frequent intervals. These were preserved in formalin acetic alcohol, and later sectioned according to the paraffin method. Petioles collected during the winter of 1929 were demineralized by treating with 25–75 per cent hydrofluoric acid for a period of 2–4 weeks.

The petioles of the various species of *Quercus* studied are very similar, varying only in minor details. Most of them are rather slender and almost cylindrical. Near the base the petiole becomes somewhat larger, and there is a slight constriction at the point of its junction with the twig. Three or more leaf traces join the vascular cylinder in the leaf gap, but in the base of the petiole these divide and diverge to form a number of separate strands surrounded by pith and cortical cells (figs. 1*a*, 3*a*). Above the abscission zone these separate strands are again united (fig. 1*b*), forming a cylinder similar to that of the stem (fig. 2).

As the separate bundles diverge, the mechanical tissue surrounding the vessels gradually decreases, until finally within the abscission zone it is practically lacking (fig. 1*c*). On the distal side of the abscission zone the mechanical tissue gradually increases, until a complete cylinder is formed. On the proximal side there is an increasing development of sclereids (fig. 3*b*), extending to the union of the petiole strands with the vascular cylinder of the stem. The sclereids are most numerous on the upper side, where in some cases they form an almost continuous sclerenchyma in the axil of the petiole strands, at which place they join the main cylinder. Crystals of calcium oxalate are abundantly distributed throughout the cells of the petiole (figs. 4–6), but they do not occur in the abscission layer during the process of abscission (figs. 4, 5, 7).

In *Quercus*, the first processes initiating development of an abscission layer take place shortly before the fall of the leaf. The first noticeable change is an increase in the density of the protoplasm in the cells of the abscission zone, and an accumulation of food in these cells. Subsequent enlargement and division of cells proceed more or less irregularly (figs. 5, 7). The distal cells of the zone elongate, and their walls swell and become distorted, in some cases being completely digested (fig. 4*a*). The inner cells of the cortex on the lower side of the petiole are the first to be affected. From this point develop-

ment extends upward, around the bundles, and outward toward the epidermis. The epidermis on the upper side is reached last, and in

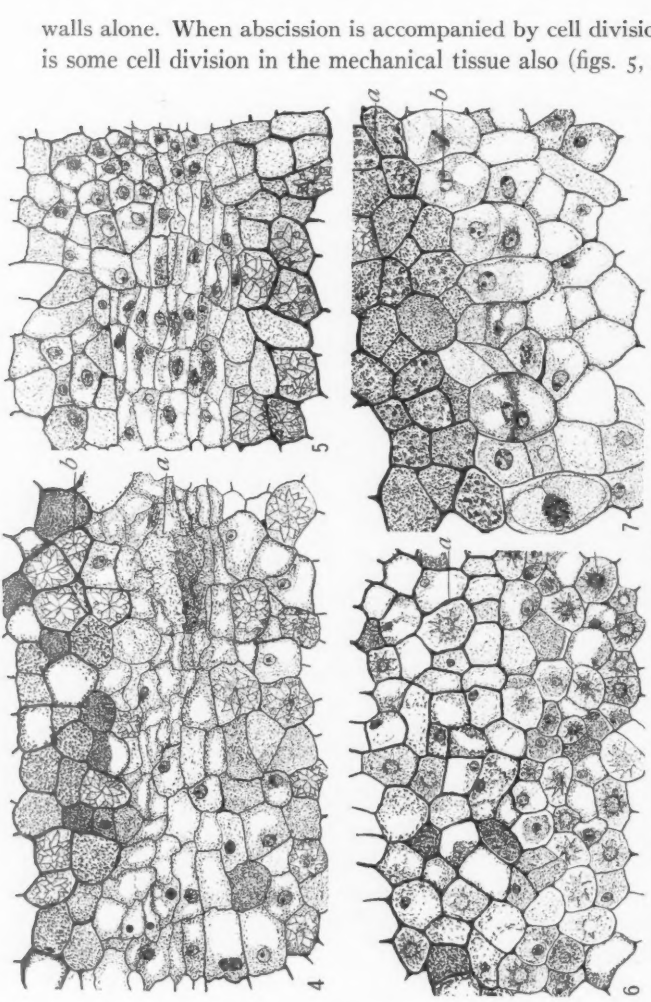


FIGS. 1–3.—Fig. 1, longitudinal diagram illustrating details of petiole in region of abscission; *a*, fibrovascular cylinder separating into separate bundles proximal to abscission zone; *b*, fibrovascular cylinder after separate bundles have reunited above abscission zone; *c*, reduction of mechanical tissue; *d*, line of abscission. Fig. 2, cross-section near point 1*b*. Fig. 3, cross-section proximal to abscission zone; *a*, separate bundle; *b*, sclereid.

many instances the tissue here is severed by mechanical force before the cell walls have been digested.

Apparently abscission may be effected in two ways, either by cell division and digestion of the cell walls, or by digestion of the cell

walls alone. When abscission is accompanied by cell division, there is some cell division in the mechanical tissue also (figs. 5, 8*a*), but



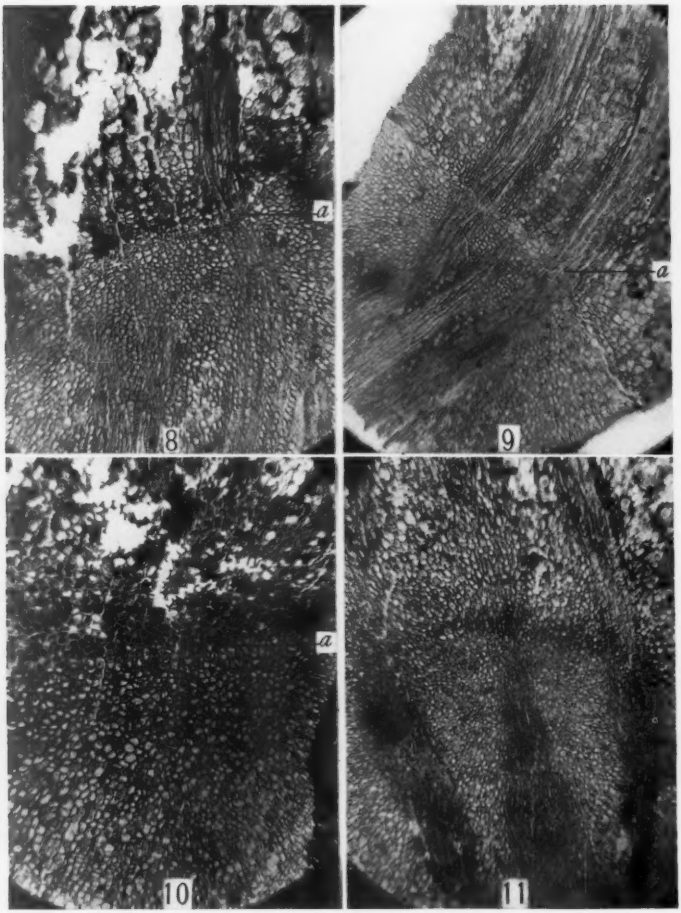
FIGS. 4–7.—Fig. 4, section of petiole through abscission layer; *a*, breaking apart of cells; *b*, aggregates of calcium oxalate crystals. Fig. 5, section of petiole showing abscission region in normally deciduous leaf. Fig. 6, section of petiole before cell division takes place in spring; *a*, ligno-suberized area characterized by heavier cell walls. Fig. 7, section of petiole of marcescent leaf showing division of cells initiating abscission layer; *a*, cells ligno-suberized during previous autumn; *b*, cells in process of division.

the new cells are much smaller than those adjacent. When abscission is not accompanied by cell division, the cell walls of the mechanical and other tissue are digested. Separation usually takes place

in the region of the middle lamella, and breaking of the petiole takes place in an abscission plane. Often the fibrovascular bundles do not break in the same plane with the cells of the other tissue, as the plane of abscission may form parallel with the bundles for the distance of a few cells before it cuts across the bundle itself.

Abscission in the various species of *Quercus* studied is often accompanied by irregular cell division. The abscission layer is developed in the more distal cells of the abscission zone, and those on the proximal side become ligno-suberized to form the protective layer over the scar. As previously stated, the cell walls in the abscission layer are digested precisely as they are in those petioles in which abscission takes place unaccompanied by a division of cells. When cell division occurs, subsequent enlargement of the new cells formed produces elongation of the petiole in this region. No divisions, therefore, occur in the vessels which, unable to elongate to conform to the increased petiole length, are subjected to stress. This stress, together with digestion of the walls of the vessels, finally produces a rupture and pulls them apart (fig. 9*a*), often before the petiole is severed from the stem. If there is no cell division, the walls of the vessels and the cell walls of the mechanical tissue are digested and broken apart simultaneously with the cell walls of the pith and cortex. The cuticle and sometimes the outer walls of the epidermal cells are the only parts that do not show alteration in the plane of the abscission layer.

Before the leaf blade is entirely dead, a greenish band, in the form of a groove, appears at the base of the petiole, in the region where a little later the line of cleavage separates the leaf from the twig. This shallow groove, extending around the petiole, apparently results from the failure of the actively dividing cells in the region of the developing abscission layer to enlarge as rapidly as do those cells lying immediately adjacent on either side. The line of cleavage usually cuts through the distal border of the groove, but the groove does not necessarily indicate the precise location of the abscission layer. In the deciduous leaves of *Quercus*, in most cases at least, the plane of cells delimited peripherally by this groove becomes ligno-suberized, either before or after leaf shedding, to form the pro-



FIGS. 8–11.—Fig. 8, photomicrograph of longitudinal section showing abscission region just before leaf fall; *a*, abscission layer through mechanical tissue. Fig. 9, abscission in normally deciduous leaf; *a*, rupturing or pulling apart of spiral vessels. Fig. 10, first cell division in formation of abscission layer in petiole; *a*, tyloses in cells of ligno-suberized tissue. Fig. 11, line of demarcation between living tissue of stem and dead ligno-suberized tissue of petiole of marcescent leaf (in which there has been no development of abscission layer).

tective layer seen in the leaf scar. This region of ligno-suberization involves 10–18 layers of cells. Tyloses form during the process of abscission, or immediately afterward in the cells of the protective layer (fig. 10*a*); these can be noticed first in the cortical cells, and finally in the vessels themselves. Such tyloses often appear before the vessels are severed.

Generally the groove is less pronounced in those leaves which die and wither on the stem-marcescent leaves, and usually they lose their green color until growth activity begins in the spring, at which time the dead leaves are abscissed. In such marcescent leaves the petiole has withered and the cells on the distal side of the plane extending through the groove are dead. In this condition the groove, if previously formed, has become so completely blended with the shrunken petiole as to be inconspicuous. The layer of cells extending across the petiole in the plane of this groove has become ligno-suberized in the same manner as those of the leaf scar already mentioned (figs. 6*a*, 11). In early spring a meristematic region develops just beneath this ligno-suberized area, giving rise to rapidly dividing cells similar to those of a cambium (figs. 4, 7, 8, 10). The thin walls of these new cells are soon digested, forming a line of cleavage that cuts off the old dry leaf (fig. 4*a*) and allows it to fall from the twig. When this abscission of the marcescent leaf occurs, the line of cleavage is formed on the proximal instead of on the distal side of the groove.

The abscission layer formed in the petioles of marcescent leaves in early spring is similar to the one formed in the petiole of a leaf that falls in autumn, the most striking difference being the more marked regularity of cell division. The cells appear like a true cambium. The new cells formed become ligno-suberized to form a protective layer over the scar left by the fall of the old dead leaf. It is apparent, therefore, that in the case of marcescence, the leaf is retained simply because an abscission layer fails to form during the autumn.

In some instances development of the abscission layer in the autumn is arrested before it is completed, and the leaf may remain on the branch for some time after the other leaves have fallen. Leaves

remaining on the trees later than January usually reveal little if any morphological evidence of the formation of an abscission layer (figs. 6, 11).

Marcescent leaves, even on the same plant, do not all fall at once, but once begun, abscission proceeds rapidly and the fall is much more uniform than is the case with leaves falling in autumn. *Quercus coccinea* is the first to become active, shedding its leaves rapidly and uniformly, since the vascular strands of the petioles are small and more easily severed. Although leaves of *Q. velutina* begin to shed at about the same time, the petioles are somewhat larger, and consequently abscission proceeds more slowly. *Q. marilandica* begins to drop its leaves shortly after, and in a manner similar to the two species just mentioned. *Q. rubra* sheds its leaves more slowly than any of the other species. This undoubtedly is due to the fact that the vascular strands of the petioles are large, and the cells in general have heavier walls. The bundles in this species do not always break in the same plane with the other cells, and so commonly leave prominent leaf traces in the leaf scar. In a great many instances a partially formed abscission layer occurs in the petioles of the larger leaves of *Q. rubra*. *Q. alba* is the last of the oaks observed to begin shedding, but once abscission begins, it proceeds rapidly. Cursory observations were made on a few species in other genera, including *Fagus grandifolia*, *Ostrya virginiana*, and *Acer saccharum* var. *nigrum*. The behavior of these is similar to that of the species of *Quercus*. The marcescent leaves of *F. grandifolia* remain on the branches long after the buds begin to swell, and in some instances until the new leaves have expanded. A single specimen of *Acer saccharum* var. *nigrum* was observed to retain its leaves until many of the new ones were almost fully expanded.

The writer wishes to express his gratitude to Drs. P. D. STRAUSBAUGH and H. S. WOLFE of the department of botany of West Virginia University for numerous suggestions and criticisms during the progress of this work.

WEST VIRGINIA UNIVERSITY
MORGANTOWN, W. VA.

[*Accepted for publication October 4, 1930*]

LITERATURE CITED

1. LEE, E., The morphology of leaf fall. Ann. Botany **25**:51–106. 1911.
2. LLOYD, F. E., Abscission. Ottawa Nat. **28**:41–52; 61–75. 1914.
3. ———, Environmental changes and their effects upon boll shedding in cotton. Ann. N.Y. Acad. Sci. **29**:1–131. 1920.
4. TISON, A., Recherches sur le chute des feuilles chez les dicotyledons. Mem. Soc. Linn. Normandie **20**:121–327. 1900.
5. VON MOHL, H., Über den Ablösungsprozess saftiger Pflanzenorgane. Bot. Zeit. **18**:273–274. 1860.

FURTHER EVIDENCE ON THE NECESSITY OF BORON FOR HEALTH IN CITRUS[1]

A. R. C. HAAS AND L. J. KLOTZ

(WITH SIX FIGURES)

A previous paper[2] described some of the physiological and anatomical changes that take place when budded citrus trees are grown for several years in pure quartz sand and are given a culture solution lacking boron.[3] Briefly these changes were: corking and splitting of the leaf veins; curling and abscission of leaves; abundant production and premature death of new growth; multiple buds; in extreme cases a splitting of the bark of twigs and trunk followed by exudation of gum, decay of roots, and an accumulation of excessive amounts of carbohydrates in affected leaves. Histological studies showed a degeneration chiefly of the cambium and phloem regions accompanied by the production of gum.

As these results were obtained from studies on a few large budded trees in sand cultures, it seemed desirable to repeat the experiments with great numbers of cultures of seedlings and cuttings. The senior author[3] has called attention to the fact that leafy lemon twig cuttings in water cultures may be supplied, from the enamel of the pans, with amounts of boron adequate for healthy growth. Likewise it was pointed out that new 12-gallon glazed earthenware containers used for sand cultures provide sufficient boron for healthy growth when boron is lacking in the original culture solution. Subsequent experiments have shown that when such containers are used for several years they fail to supply boron in amounts adequate for normal growth, as was evidenced by the appearance of all the symptoms typical of boron starvation. When boron is supplied to such cultures the symptoms of deficiency rapidly disappear. The badly affected

[1] Paper no. 232, University of California Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

[2] HAAS, A. R. C., and KLOTZ, L. J., Some anatomical and physiological changes in citrus produced by boron deficiency. Hilgardia 5:175–197. 1931.

[3] HAAS, A. R. C., Boron as an essential element for healthy growth of citrus. BOT. GAZ. 89:410–413. 1930.

leaves show but little improvement in appearance when boron is supplied, but absciss less prematurely.

The 2-quart Mason jars in our glasshouse experiments have been in use for over ten years. During the greater portion of this time the jars have been used for experiments with citrus seedlings or cuttings, and although no boron was added to the culture solution, not until about the tenth or present year have any symptoms of boron deficiency been evident. Now, however, practically every plant in each of 80 or more jars shows symptoms of boron deficiency. Figs. 1 and 2 show the effects on leaves and roots. The amount of boron in the seed or in the leaves and twig of the leafy cuttings is sufficient to support normal growth for only a short time.

Leafy lemon cuttings that have grown several years in Swedish enameled mixing bowls of 6-liter capacity, containing a culture solution from which boron was omitted, are now gradually showing more of the symptoms of boron deficiency. Whenever boron is added to the culture solution in concentrations of 0.1 or 0.2 p.p.m., the new growth shows marked improvement regardless of whether the citrus grown is of seedling or cutting origin. The results of our experiences show that it is essential that the glassware, earthenware, or enamelware containers be in use several years prior to their being used in experiments on boron deficiency; otherwise it might be concluded that boron is not an essential element unless the cultures are allowed to grow for several years.

In addition to ordinary leafy twig cuttings, various grafted combinations[4] of leafy twig cuttings were grown in aged 2-quart Mason jars in a culture solution from which boron was omitted. In making these combinations the three leaves of the leafy cutting used as the stock were removed prior to rooting the stock in sand, and only the cutting represented by the scion was allowed to produce new leaves.

There were seven sour orange (*Citrus aurantium* Linn.), thirteen Rough lemon (var. of *C. limonia* Osbeck), two Spanish bitter orange (var. of *C. aurantium* Linn.), and seven Lisbon lemon (var. of *C. limonia* Osbeck), 3-leaf cuttings. The cutting combinations of scion on stock consisted of two Valencia orange (*C. sinensis* Osbeck) on

[4] These cuttings were made by Dr. F. F. Halma of the University of California Citrus Experiment Station.

Valencia orange, four Valencia on Eureka lemon (var. of *C. limonia* Osbeck), two Valencia orange on sour orange, four Eureka lemon

FIG. 1.—Leaves from leafy grapefruit cutting as scion on Eureka lemon cutting as stock: upper surface showing corky split veins of leaves from boron-deficient cultures (bud in axil of attached leaf developed slightly and then died).

on sour orange, three Rough lemon on Eureka lemon, three Marsh grapefruit (*C. maxima* (Burm.) Merrill) on Eureka lemon, one sour orange on sour orange, and two sour orange on Rough lemon. In

each of these, and in many additional cultures designed for other purposes, whenever boron was omitted for several months the leaves showed corky and split veins, multiple buds, cessation of new growth of tops and roots, bulbous enlargement of root tips, and eventual decay of much of the root system. No gum formation has as yet been observed in any of these young cultures. It was evident that insufficient boron was being obtained from the aged Mason jars or as an impurity from the salts used in preparing the culture solutions.

FIG. 2.—Effect of boron-deficiency on roots of sour orange cuttings in water culture: left, affected roots showing stunted bulbous tips and considerable decay; right, normal root system.

The addition of boron as boric acid in an amount sufficient to make a concentration of 0.1 to 0.2 p.p.m. in the culture solution brought about unusually rapid recovery. Fig. 3, showing cultures of lemon seedlings of the same age, demonstrates the necessity for boron and the recovery following the addition of boron. Figs. 4–6 show similar effects with 3-leaf cuttings and with grafted combinations of cuttings.

It has been shown in our previous paper[5] that where boron was omitted from the culture solution, sugars accumulated in the leaves. This was associated with disintegration of the conducting tissue. Upon the addition of boron to these cultures, there was rapid growth

[5] See footnote 2.

of new conducting tissue, followed by a decrease in the sugar content of the leaves as recovery progressed.

In the present investigation with sour orange cuttings, determinations of total sugars plus starch in the mature leaves of boron-deficient cultures showed approximately double the amount found in mature leaves of cultures supplied with boron, the values being re-

FIG. 3.—Lemon seedlings showing: right, symptoms of typical boron-deficiency; left, recovery showing normal growth following addition of boron.

spectively 11.67 and 5.96 per cent of the dry matter. These results with cuttings in water culture confirm the previous results with budded trees in sand culture. The early resumption and the rapidity of growth, following the addition of boron to cultures previously deficient in this element, may be a consequence of the rapid movement of this large supply of carbohydrates following formation of new conducting tissue.

FIGS. 4–6.—Fig. 4, sour orange cuttings showing: left, curled leaves with corky split veins, typical of boron deficiency; right, new healthy growth following addition of boron (all affected leaves formerly present in culture on right have abscissed). Fig. 5, combination of leafy cutting of Rough lemon as scion on Eureka lemon cutting as stock: right, defoliation as result of boron deficiency; left, new growth following addition of boron to culture previously deficient in this element. Fig. 6, combination of leafy cutting of Eureka lemon as scion on sour orange cutting as stock: right, defoliation as result of boron deficiency; left, new growth following addition of boron to culture solution previously deficient in this element.

To throw further light on the causes for the rapid recovery of boron-deficient citrus when given boron, the relative diastatic activity of mature leaves of boron-deficient and boron-supplied cultures was determined. Five hundred-mg. samples of the dry leaf powder and 25 cc. samples of a 1 per cent starch solution were placed in 150 cc. Erlenmeyer flasks and incubated 12 hours at 40° C. The suspension was then filtered and the reducing sugars of the filtrate determined. Using the same procedure, blank determinations of 500-mg. samples of leaf powder in 25 cc. of distilled water were made at the same time. The results obtained were subtracted from those of the first set in order to correct for differences in the intrinsic reducing power of the leaves. The leaves from the boron-deficient cultures showed a slightly greater diastatic activity than those receiving boron in the culture solution, the results being in the ratio of 150 to 138.5 in the case of the sour orange cuttings, and 131 to 120 in the case of the cutting combination of Eureka lemon scion on Rough lemon stock. These results suggest a further reason for the rapid recovery of formerly boron-deficient citrus, and the simultaneous rapid decrease in the carbohydrate content of the leaves.

UNIVERSITY OF CALIFORNIA
CITRUS EXPERIMENT STATION
RIVERSIDE, CALIF.

[*Accepted for publication October 13, 1930*]

A NEW METHOD FOR DETERMINING THE PROPORTION OF THE LENGTH OF A TRACHEID THAT IS IN CONTACT WITH RAYS[1]

ALFRED J. STAMM

(WITH ONE FIGURE)

In the progress of some research work, it became desirable to know just what proportion of the length of a tracheid along the radial face of a piece of softwood is in contact with rays. Obtaining such information through direct measurement of tangential microtome sections, or of the corresponding photomicrographs, is an extremely tedious operation, since a fair average value then necessitates a great number of measurements. This reason made preferable a simplified indirect method that gives an average value directly, and, more important, substitutes the simple operation of counting the structural elements for the laborious and uncertain one of measuring them.

The determinations were made on transverse- and tangential-section photomicrographs that had been enlarged fifty times. For the tangential sections, the number of double walls of tracheids extending across the photomicrograph along the line of intersection of any transverse plane, usually over an actual distance of 0.30 cm., were counted as well as the number of rays cut by this line; for this purpose the "double walls" included the pairs of single walls separated by rays as well as those actually in contact. The number of rays so intersected, divided by the total number of double tracheid walls, gives the average ratio of length of tracheid and rays in contact to the total length of both tracheid-ray contact and tracheid-tracheid contact along the radial faces of the tracheids considered, providing the number counted is not too small. This relationship may be stated in somewhat different terms as follows: The probability of any transverse plane that intersects a tracheid touching a ray cell

[1] Contribution from the Forest Products Laboratory, Forest Service, U.S. Department of Agriculture, maintained at Madison in cooperation with the University of Wisconsin.

will vary directly with the ratio of the combined heights of all rays that are in contact with the tracheid to the total tracheid length.

As a check upon the validity of this relationship, a few determinations were made with the direct-measurement and the count method on the same photomicrographs. In order to increase the accuracy for the direct-measurement method, tangential-section photomicrographs with an enlargement of 300 times were used. The sum of the heights of all the rays in the direction of the tracheid lengths and the sum of all the tracheid wall lengths making up the photomicrograph section were determined. Fig. 1 shows a tangential photo-

TABLE I

RATIO OF LENGTH OF TRACHEID-RAY CONTACT TO TOTAL TRACHEID-WALL CONTACT BY BOTH THE COUNT AND THE DIRECT-MEASUREMENT METHODS, FOR PICEA SITCHENSIS

SPECIMEN NO.	COUNT METHOD			DIRECT-MEASUREMENT METHOD		
	Average number of		Ratio of rays to tracheid walls	Total height of rays (cm.)	Total length of tracheid walls (cm.)	Ratio of ray to tracheid length
	Rays crossed	Tracheid walls crossed				
1	3.22	16.0	0.201	74.5	367	0.203
	3.48	17.0	0.205	81.9	403	0.203
	2.78	16.0	0.174	66.4	380	0.175
2	3.22	19.9	0.162	77.8	476	0.163
	2.82	17.8	0.158	65.5	426	0.154
	3.17	17.6	0.180	69.9	400	0.175

micrograph of *Picea sitchensis*[2] with the heights of the rays indicated, and the first line of table I shows the results obtained from this section. Because of the relatively few tracheids and the smaller number of rays intersected by a single transverse line, counts of the rays cut by a number of transverse lines were made at intervals of 1 cm. on the original photomicrographs (fig. 1), and the average was taken so as to give more accurate results. Table I gives the results for three different photomicrographs of each of two different specimens. The ratios of length of tracheid-ray contact to total tracheid-wall contact obtained by the two methods agree very well.

Although fig. 1 and table I show that the results obtained by the

[2] Names of species in this article follow SUDWORTH, G. B., Check list of the forest trees of the United States, their names and ranges. U.S. Dept. Agric. Misc. Circ. 92.

two methods are comparable, they do not show the real saving in labor of the count method. When the counts are made upon enlargements of lower power, which was done for the data given in table II, the results demonstrate that the count made across a single intersecting line gives a fair average value (for 75 to 100 tracheids), and that a single line is therefore sufficient. It is obvious that such a count can be made much more readily than the measurement in the

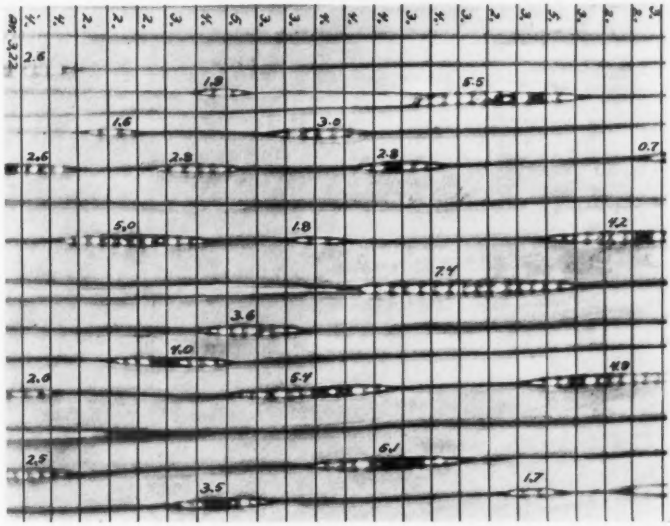


FIG. 1.—Cross-section of softwood, ruled to show statistical principle underlying the new method, which in practice needs only a count made along a single straight edge; ×150.

direct method of the total height of the rays touching a single tracheid.[3] In general the count data should also give a better average value, since there is more chance of the properties of a single tracheid varying from the average than the properties of the great number of tracheids along the line of intersection.

Data can also be obtained by the count method from transverse sections. In them the rays are continuous, and can be counted easily.

[3] A recent article (CLARKE, S. H., Forestry 4:42. 1930) describes a recording micrometer used in conjunction with a projection apparatus that would greatly simplify the direct-measurement method, but still the indirect method should be less laborious.

TABLE II

PROPORTION OF LENGTH OF TRACHEID IN CONTACT WITH RAYS DETERMINED BY COUNT METHOD FOR VARIOUS SOFTWOODS ON PHOTOMICROGRAPHS OF 50 TIMES ENLARGEMENT

NAME OF SPECIES	TRANSVERSE SECTION				TANGENTIAL SECTION				AVERAGE OF BOTH GROUPS OF RATIOS
	Distance examined (cm.)	No. tracheids	No. rays	Ratio	Distance examined (cm.)	No. tracheids	No. rays	Ratio	
Abies amabilis	.3	72	15	.208	.3	72	15	.208	.204
	.3	72	14	.194	.3	75	16	.213	
	.3	75*	15	.200	.3	73	15	.205	
	.3	70	14	.200	.3	70	14	.200	
Abies balsamea	.3	110	19	.173	.3	113	19	.168	.168
	.3	111	18	.162	.3	114	20	.175	
	.3	114	19	.167	.3	109	18	.165	
Abies concolor	.3	86	14	.163	.3	89	15	.169	.177
	.3	88	15	.170	.3	88	17	.193	
	.3	89*	16	.179	.3	84	16	.190	
Abies magnifica	.3	74	20	.270	.3	72	20	.278	.278
	.3	75	21	.280	.3	70	19	.271	
	.3	79	22	.279	.3	73	21	.288	
Abies nobilis	.3	75	12	.160	.3	94	14	.149	.154
	.3	78	12	.154	.3	99	15	.152	
	.3	81	13	.160	.3	101	15	.149	
Chamaecyparis lawsoniana	.3	109	9	.083	.3	112	11	.098	.089
	.3	106	10	.094	.3	112	10	.089	
	.3	110	9	.082	.3	107	10	.093	
Chamaecyparis thyoides	.3	106	12	.113	.3	101	12	.119	.122
	.3	105	12	.114	.3	105	14	.133	
	.3	106	14	.132	.3	105	13	.124	
Juniperus virginiana	.28	124	16	.129	.3	131	16	.122	.118
	.28	127	15	.118	.3	129	15	.116	
	.28	125	14	.112	.3	126	14	.111	
Larix laricina	.3	104*	25	.240	.3	103	26	.252	.250
	.3	100	24	.240	.3	94	26	.277	
	.3	97	23	.237	.3	98	25	.255	
Larix larix	.3	83	19	.229	.3	95	21	.221	.229
	.3	90*	21	.233	.3	91	22	.242	
	.3	86	20	.233	.3	96	21	.219	
Larix occidentalis	.3	83	15	.181	.22	53	10	.189	.187
	.3	87*	16	.184	.22	50	10	.200	
	.3	84	16	.190	.22	53	10	.189	

* Summerwood.

TABLE II—*Continued*

Name of species	Transverse section				Tangential section				Average of both groups of ratios
	Distance examined (cm.)	No. tracheids	No. rays	Ratio	Distance examined (cm.)	No. tracheids	No. rays	Ratio	
Libocedrus decurrens	.3	121	16	.132	.3	113	16	.142	.141
	.3	122	17	.139	.3	116	18	.155	
	.3	125	17	.136	.3	114	16	.140	
Picea glauca	.3	91	20	.220	.3	95	21	.221	.221
	.3	95*	23	.242	.3	100	22	.220	
	.3	91	18	.198	.3	107	24	.224	
Picea mariana	.3	101	16	.158	.3	97	16	.165	.167
	.3	102	18	.176	.3	103	17	.165	
	.3	110*	19	.173	.3	102	17	.167	
Picea sitchensis	.3	100	20	.200	.3	98	17	.173	.185
	.3	108	18	.167	.3	99	18	.182	
	.3	100	18	.180	.3	92	19	.207	
Pinus banksiana	.3	116	24	.207	.3	109	22	.202	.205
	.3	121*	25	.207	.3	104	23	.221	
	.3	116	23	.198	.3	109	21	.193	
Pinus contorta	.3	106	14	.132	.3	107	14	.131	.139
	.3	112*	16	.143	.3	102	14	.137	
	.3	105	15	.143	.3	103	15	.146	
Pinus echinata (wide growth rings)	.3	80	13	.163	.3	83	12	.145	.152
	.3	83*	14	.169	.3	84	11	.131	
	.3	88	14	.159	.3	83	12	.145	
Pinus echinata (narrow growth rings)	.3	80	13	.163	.3	78	13	.167	.152
	.3	88*	14	.159	.3	79	12	.152	
	.3	83	11	.133	.3	78	11	.141	
Pinus lambertiana	.3	64	5	.078	.3	65	6	.092	.086
	.3	69*	5	.072	.3	68	6	.088	
	.3	65	5	.077	.3	65	7	.108	
Pinus monticola	.3	99	8	.081	.3	92	8	.087	.089
	.3	92*	9	.098	.3	94	8	.085	
	.3	93	8	.086	.3	95	9	.095	
Pinus ponderosa	.3	101	15	.149	.3	94	13	.138	.144
	.3	109	17	.156	.3	103	14	.136	
	.3	109	16	.147	.3	104	14	.135	
Pinus resinosa	.3	104	16	.154	.3	104	13	.125	.141
	.3	100	14	.140	.3	96	12	.125	
	.3	99*	16	.162	.3	92	13	.141	

* Summerwood.

TABLE II—*Continued*

Name of species	Transverse section				Tangential section				Average of both groups of ratios
	Distance examined (cm.)	No. tracheids	No. rays	Ratio	Distance examined (cm.)	No. tracheids	No. rays	Ratio	
Pinus taeda	.3	95	18	.189	.3	101	16	.158	.170
	.3	92*	18	.196	.3	96	14	.146	
	.3	94	17	.181	.3	105	16	.152	
Sequoia sempervirens (small cells)	.3	117	19	.162	.3	116	17	.147	.145
	.3	126*	19	.151	.3	116	16	.138	
	.3	127	17	.134	.3	118	16	.136	
Sequoia sempervirens (large cells)	.3	62	10	.161	.3	59	10	.169	.165
	.3	60*	10	.167	.3	61	10	.164	
	.3	60	10	.167	.3	56	9	.161	
Sequoia washingtoniana	.3	99	20	.202	.3	98	18	.184	.185
	.3	97	18	.186	.3	102	18	.176	
	.3	100*	17	.170	.3	103	20	.194	
Thuja occidentalis	.3	125	11	.088	.3	113	11	.097	.095
	.3	120	11	.092	.3	116	11	.095	
	.3	124	13	.105	.3	120	11	.092	
Thuja plicata (wide growth rings)	.3	130	14	.108	.3	105	14	.133	.120
	.3	129*	16	.124	.3	117	14	.120	
	.3	129	13	.101	.3	113	15	.133	
Thuja plicata (narrow growth rings)	.3	106	11	.104	.3	111	15	.135	.126
	.3	108*	13	.120	.3	100	14	.140	
	.3	103	13	.126	.3	97	13	.134	
Tsuga canadensis	.3	105*	21	.200	.3	131	20	.153	.178
	.3	94	18	.191	.3	129	21	.163	
	.3	97	19	.196	.3	128	21	.164	
Tsuga heterophylla	.3	94	14	.149	.3	90	14	.156	.158
	.3	97*	16	.165	.3	96	16	.167	
	.3	97	15	.155	.3	88	14	.159	

* Summerwood.

Dividing the number of rays that any tangential line intersects by the number of double tracheid walls along that line again gives the ratio of tracheid-ray contact length to that of total tracheid-wall. A comparison of results obtained on transverse- and tangential-section photomicrographs is given in table II, three separate counts being made on different parts of each photomicrograph. The transverse-section data give further a comparison between the values of the ratio for summerwood and for springwood.

Although the results given in table II are insufficient to permit drawing general conclusions regarding the ratio of tracheid-ray contact length to total tracheid-wall contact length, because only one or two specimens from each species have been included, they point to several interesting relationships. The ratio is practically the same for both summerwood and springwood, indicating that there is little relationship between the local density of the wood and this ratio. The results show also that values for the ratio obtained from different parts of the same small specimen are substantially constant. This is not surprising, since each value is an average for a great number of tracheids and thus should at least approximate the over-all average. The agreement between the results for the transverse and the tangential sections is good, and is all the more indicative because the photomicrographs examined were picked from the files at random, and the sections may not have come from the same block. In three instances results are given for two different sets of specimens of the same species. The agreement for *Pinus echinata* is far better than can generally be expected, while for *Sequoia sempervirens* and *Thuja plicata* the agreement is good, indicating that the variation within a single species may not be great. Further, the variation between the values for different species of the same genus in general is less than the over-all variation.

Summary

1. A simple method has been suggested for determining the ratio between the length of tracheid-ray to total tracheid-wall contact that requires only the counting of structural elements.

2. A comparison between the results obtained with this method and with the direct-measurement method is given.

3. The values for this ratio obtained for all the different species of softwood examined range from 0.072 to 0.288. Values of the ratio for a given species vary considerably less, and, for different parts of the same specimen, still less. There is no significant difference between the values for summerwood and for springwood.

FOREST PRODUCTS LABORATORY
U.S. DEPARTMENT OF AGRICULTURE
MADISON, WIS.

[Accepted for publication October 27, 1930]

CURRENT LITERATURE

BOOK REVIEWS

Taxonomy of flowering plants

This journal has already noted texts in plant taxonomy by DIELS and by HITCHCOCK (BOT. GAZ. **81**:342. 1926), and also by SWINGLE (*ibid.* **86**:115. 1928). The appearance of another work in this field is now recorded, the noteworthy text by JOHNSON.[1] This work is monumental in many respects, although it abounds in chronological, orthographic, lexicographic, and other types of inaccuracies to an extent greater than can be justified, even in a first edition. The author displays a broad and balanced perspective, and appears to have kept foremost in mind the needs of students and the demands of taxonomic pedagogy. His style is dignified and conservative, yet as interesting and attractive as the limitations of the subject matter permit. An outstanding feature of the volume is the surprising wealth of original drawings. These number several thousand, grouped into nearly five hundred aggregations modestly called figures, but which might with greater propriety have been treated as full-page plates. These illustrations were drawn by the author himself, and combine to an exceptional degree morphological accuracy with artistic excellence. They abound in glimpses of the more intimate or even cryptic details of floral structure. It is safe to predict that for many years to come these alone will be sufficient to insure the use of JOHNSON'S book by teachers and students, not only in plant taxonomy but also in allied branches of botany.

Part I consists of 150 pages, and is devoted to flower analysis and fundamental principles. In chapter I, the science of taxonomy is treated briefly in historical retrospect. Subsequent chapters deal with such subjects as nomenclature, genus and species, phylogeny, homology and analogy, the flower, fruit, seed, seedling, inflorescence, and vegetative characters. Part II opens with chapter X and includes the remainder of the book. It is devoted to systematics. Chapter X contains many valuable generalizations and discusses the approach to classification. It is followed in chapter XI with various practical suggestions on species and specific distinctions, genera, generic characters and distinctions, fruit and seed characters, leaf characters, comparisons, construction of keys, field studies, and collecting for a herbarium. The rest of the text proper, which is by far the larger part of the entire book, is given over to the *Dicotyledones* and *Monocotyledones*, group by group. Here the author has drawn freely upon WETTSTEIN, ENGLER and PRANTL, BENTHAM and HOOKER, and other writers for technical material pertaining to the different groups. The volume concludes

[1] JOHNSON, A. M., Taxonomy of the flowering plants. pp. xxi+864. *figs. 478*. Century Co., New York. 1931.

with a glossary and a bibliography. The glossary is somewhat full for a work of this size, but a cursory inspection shows various points meriting criticism. Thus, *amplexicaule* is the spelling given to a supposedly English adjective, while Gray's Manual, the Century Dictionary, etc., prefer *amplexicaul; decussate* is defined as "alternating in two rows, in zigzag manner, on opposite sides of the stem," which is hardly to be reconciled with "alternating in pairs at right angles" as in Gray's Manual, the Century Dictionary, and other authoritative works. The bibliography is planned in a helpful way, since it is subdivided into sections dealing with floras, manuals, and special treatises; manuals and handbooks of trees and shrubs; works on grasses, including cereals; works of a popular nature; works on teratological phenomena; works on ecology, phytogeography, natural history; works on forests and forestry; works on geology and paleontology; histories of botany; botanical periodicals in English; ecological journals; additional works (textbooks) on ecology; and recent textbooks on systematic botany. The usefulness of the bibliography is impaired, however, by many inclusions of insignificant works and omissions of important ones, not to mention mistakes in dates, proper names, etc. Surely a second edition can be made to represent taxonomy with much greater fidelity if it shall first have been given a vigorous and detailed re-editing. Such re-editing would prove particularly valuable if to the task could be brought a taxonomist of much practical experience in herbarium and library research, as distinguished from that in class-room and field work.—E. E. SHERFF.

Tall-grass prairie in Nebraska

An unusually thorough and valuable description of some of the climatic and soil conditions of the Nebraskan tall-grass prairie is made available by WEAVER and HIMMEL.[2] Observations and results of instrumentation are presented, derived from a study that extended from 1915 to 1928, for an upland station and for one in the lowland, both in the vicinity of the city of Lincoln. A brief characterization of the two samples of climax vegetation is followed by descriptions of the two soils, including the usual data from mechanical and chemical analyses. Then the following fluctuating environmental conditions are described, with tables and graphs: amount of precipitation and its seasonal distribution; soil-moisture content at several depths (expressed as gravimetric percentages above the hygroscopic limit); air temperature; soil temperature (at several depths); air humidity (expressed as relative humidity); wind movement (treated only superficially); and evaporation (nearly complete weekly records of water loss from standardized white cylindrical porous porcelain atmometers for the summers of 1916, 1917, 1919, 1920, 1921, 1922, 1924, 1925 and 1926). Although some of the methods of instrumentation and of computation and integration might conceivably be improved, it is remarkably fortunate that routine

[2] WEAVER, J. E., and HIMMEL, W. J., The environment of the prairie. Bull. no. 5. Conservation and Survey Division, Univ. of Nebraska. 1931.

procedure was almost wholly maintained throughout these many seasons. Consequently the extensive data form a nearly homogeneous series and give a presumably fair picture of the conditions for eastern Nebraska in the present climatic period. Differences between the different summers are discussed. Physiologists and ecologists, and students of applied botany also, may note that our present lack of any well authenticated and feasible procedure for evaluating solar radiation as a climatic feature is tacitly emphasized, for that important climatic condition is not considered at all.

The paper is primarily descriptive and the discussions are not at all exhaustive. An important general conclusion, in which most students of ecological climatology will probably concur readily enough, is that the amplitudes of the natural fluctuation of the non-water conditions of the region in question, from week to week, and from summer to summer, are not sufficiently great to be markedly influential on this type of vegetation, but that the natural fluctuation of the moisture conditions of air and soil do have amplitudes wide enough to bring these conditions almost into the category of limiting factors. Slight differences in moisture conditions appear to account for the notable vegetational differences between upland and lowland prairie vegetation in general, and between the performance of the component plants in different years. If the climate were to become only slightly drier or wetter the vegetation would probably show prompt and conspicuous adjustment, but it would probably require much more pronounced change in temperature conditions to bring about notable alteration in the vegetational aspect. This is in accord with indications that may be derived from a vegetation map of the United States.—B. E. LIVINGSTON.

Handbook of plant nutrition and fertilizer science

A work of unusual magnitude and outstanding value in the field of plant nutrition has been prepared under the able leadership of HONCAMP,[3] the director of the Rostock Experiment Station. The work consists of two large volumes, one on plant nutrition, the other on fertilizer science. It is difficult to do justice to a great contribution such as this in a limited review.

Realizing the specialized development of the field covered, HONCAMP secured the collaboration of an extensive group of experts, fifty of whom were enlisted in the effort. Each important section has been prepared by one whose experience and interests best fitted him for the task. The work is well illustrated, with 90 figures in the first volume and 285 in the second.

Volume I contains ten chapters. It opens with an interesting history of plant nutrition, from the time of ARISTOTLE down to HELLRIEGEL'S famous experiments. The second chapter deals with the constituents and composition of the plant body, and the third with the cycle of the main nutrient elements in

[3] HONCAMP, F., Handbuch der Pflanzenernährung und Düngerlehre. Vol. I, Pflanzenernährung. 8vo. pp. xv+945. Vol. II. Düngemittel und Düngung. 8vo. pp. xii+919. Julius Springer, Berlin. 1931.

nature. Chapter IV is devoted to the physiology of metabolism in plants. The next chapter considers the soil as the habitat and nutrient reservoir for plant growth. Chapter VI deals with the yield laws, chapter VII with methods of investigation, water and sand cultures, etc., and chapter VIII with methods of field investigations. The last two chapters discuss the evaluation of fertilizer experiments and the determination of nutrient deficiencies of soils. Here one finds illuminating discussions of MITSCHERLICH'S methods and the seedling method of NEUBAUER and SCHNEIDER. As this is discussed by NEUBAUER himself, it is of course an authoritative account.

Volume II presents the fertilizer problems in nine chapters, the first of which is again a general review of the field, and the second an account of naturally occurring fertilizer materials. Chapters III and IV consider the manufactured fertilizers and their application. Chapter V discusses the specific fertilizer requirement of certain crop plants, root crops, grains, legumes, oil and fiber plants, meadows and pastures, vegetables, vineyards and orchards, hops, tobacco, etc. Chapter VI is devoted to forest fertilizer problems, chapter VII to those of moor and heath, and chapter VIII to those of ponds. The last chapter considers the relation of fertilizers to plant disease and insect problems, and to the control of weeds. There is an enormous amount of valuable information gathered into these volumes, and the work will prove very helpful as a reference and guide to the present status of plant nutrition studies. Those who are fortunate enough to be able to use them will appreciate the immense amount of time and labor which have been required of the editor, collaborators, and publishers in making such a vast source of information available to the investigator.—C. A. SHULL.

Soil microorganisms

An interesting book has been written by WAKSMAN and STARKEY[4] describing the microbes of the soil, and giving their relation to soil processes and plant growth. Botanists know all too little about the microscopic organisms of the soil. As brought out by the authors, the soil is not just an inert mixture of disintegrated rock and organic matter, but is teeming with a microscopic population of plants and animals. The activities of these microorganisms are absolutely essential for the growth of higher plants, and indirectly for the development of all higher life, both plant and animal. By way of illustration, to be available for plant growth, nitrogen must be present in the soil in the form of nitrates or ammonium salts; but nitrogen in these forms is never present in the soil in amounts of more than a few pounds per acre. Microorganisms of the soil are constantly changing the organic nitrogen to ammonium salts and nitrates, thus keeping in circulation some of the essential elements.

The book is divided into ten chapters. Chapter I is concerned mainly with

[4] WAKSMAN, S. T., and STARKEY, R. L., The soil and the microbe: an introduction to the study of the microscopic population of the soil and its rôle in soil processes and plant growth. 8vo. pp. xi+260. *figs. 85.* John Wiley & Sons, New York. 1931.

the processes of soil formation and plant nutrients. Chapters II and III discuss the microbes of the soil and their distribution and activity. Chapter IV considers the work of the microorganisms in decomposing the organic matter of the soil. Chapters V, VI, and VII are concerned with the rôle of microorganisms in the transformation of the soil nitrogen and of the mineral substances. In chapter VIII various interrelationships between the plant and the microorganisms of the soil are considered, including nitrogen fixation, nodule formation by legumes, mycorhiza, etc. Chapter IX deals with the effects of various factors, such as organic matter, cultivation, liming, and reaction, in modifying the soil population. Chapter X is of the nature of a summary, giving the importance of microbes in soil fertility.

The book is not intended as an exhaustive treatise of the subject, but as a compact description of the microorganisms of the soil and their activity. It serves this purpose admirably. It is written in a clear, convincing style. One characteristic contributing to its clarity and readability is that when a point is in controversy, the theory backed by the most evidence is given, without much reference to other possible theories. While the research worker would probably desire a fuller discussion of other possible theories, it must be admitted that the plan adopted has its value. A list of the most important books is given at the end of each chapter. No citations are referred to by number in the text of the book. The authors have given an excellent description of the microorganisms of the soil and their relationships to soil fertility.—S. V. EATON.

Plant ecology

Four years have elapsed since the publication of McDOUGALL'S textbook in plant ecology, and now the second edition has appeared.[5] The order of presentation is precisely the same as that of the first edition, and there has been little change in the textual material. About one-half of the chapters are identical with those of the previous edition, and in the other chapters the changes were made by inserting a brief paragraph or two to state some additional facts. The most extensive modification of this kind occurs in chapter XII, where the author has added two paragraphs dealing with atmospheric humidity. There are five new illustrations, and a number of new references have been placed in the lists at the end of each chapter. In his preface to the new edition the author states that "the material has been carefully revised in order to bring it up to date," and it is therefore somewhat disappointing to find no hint of some of the more recent work; for example, no reference has been made to MAXIMOW'S[6] interpretation of xerophytism.—P. D. STRAUSBAUGH.

[5] McDOUGALL, W. B., Plant ecology (2d ed). pp. xii+338. Lea & Febiger, Philadelphia. 1931.

[6] MAXIMOW, N. A., The physiological nature of drought resistance of plants. Proc. Internat. Congress Pl. Sci. Ithaca, New York. 1926. 2:1169–1175. 1929.